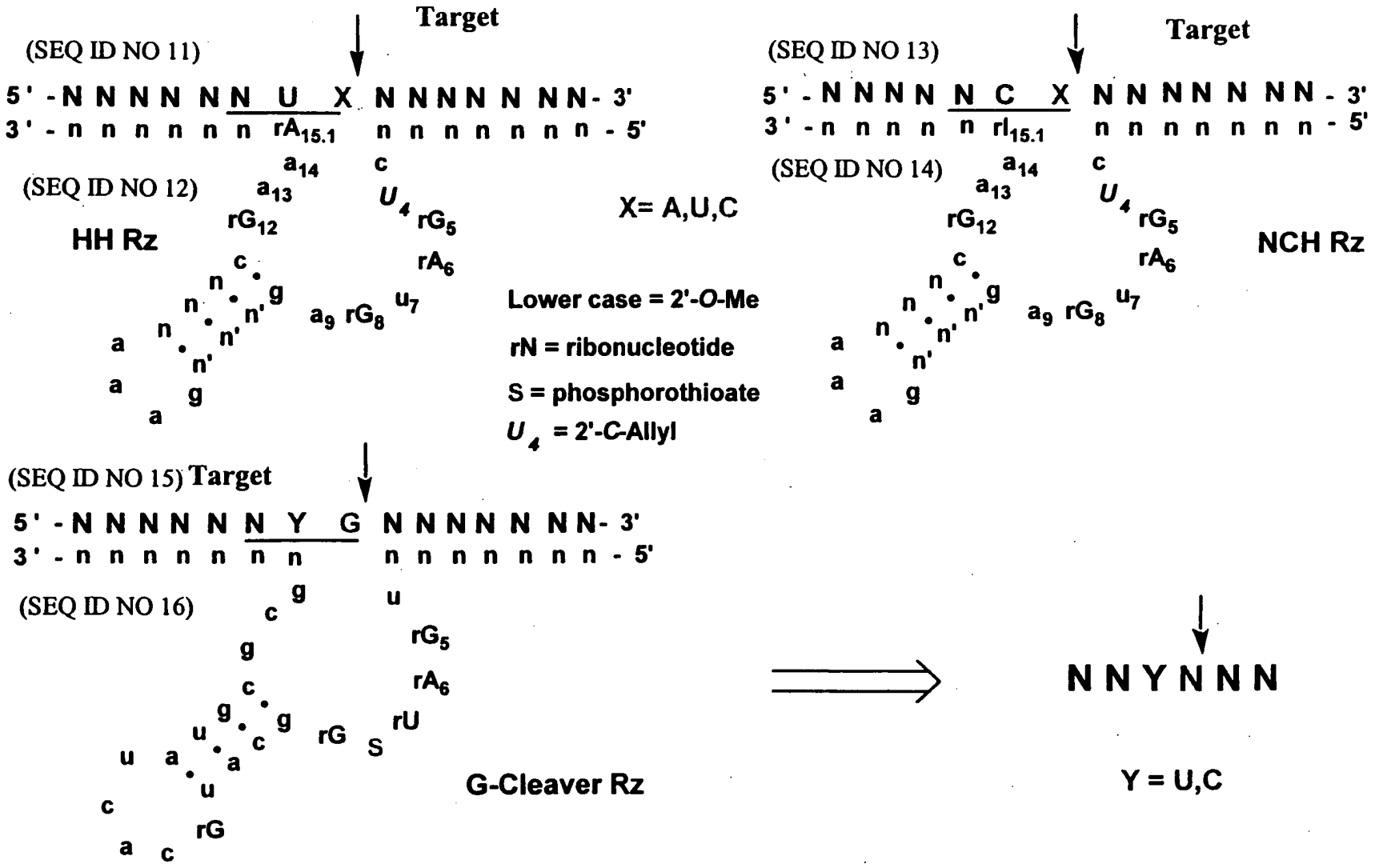
20220 "T9295007

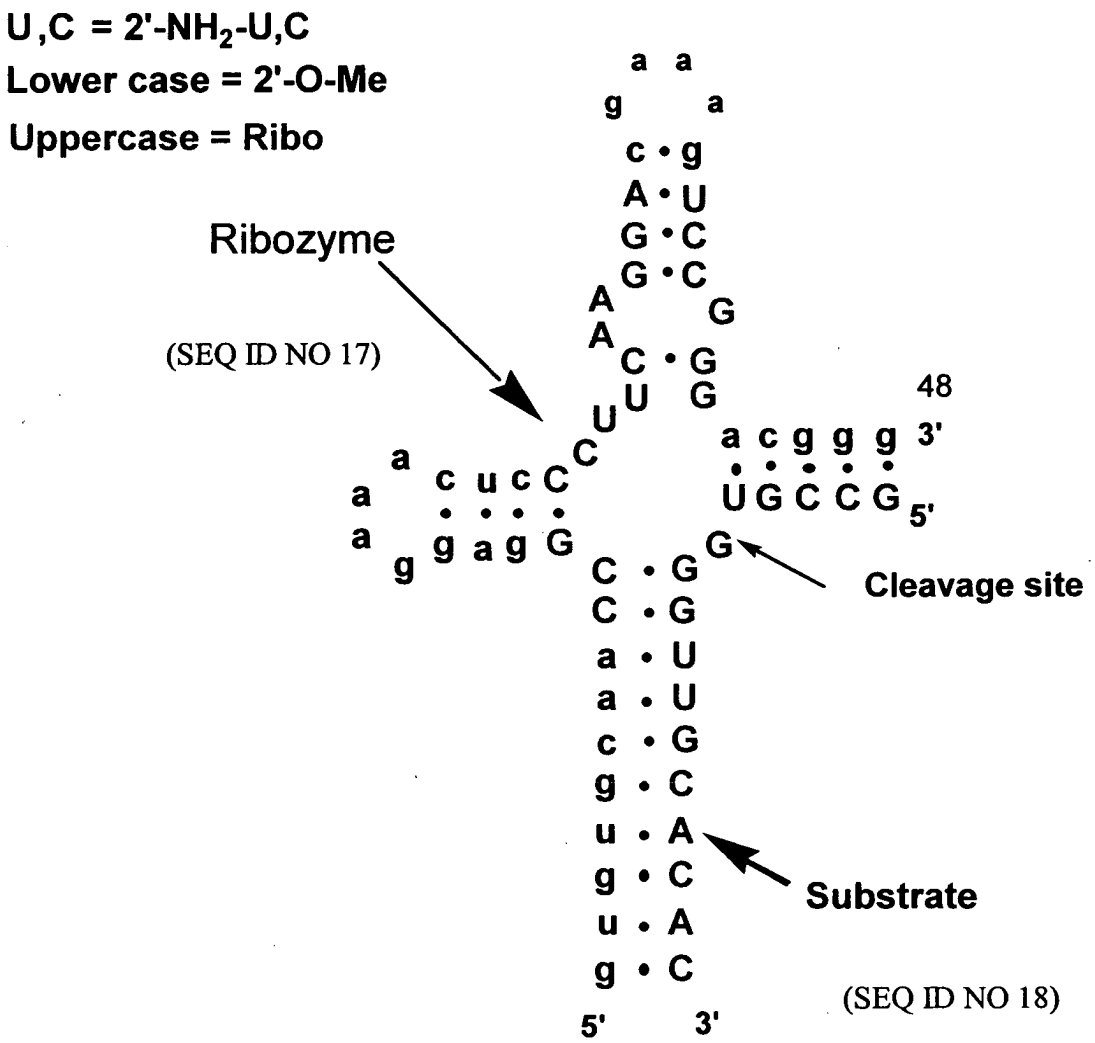
Figure 1: Examples of Nuclease Stable Ribozyme Motifs



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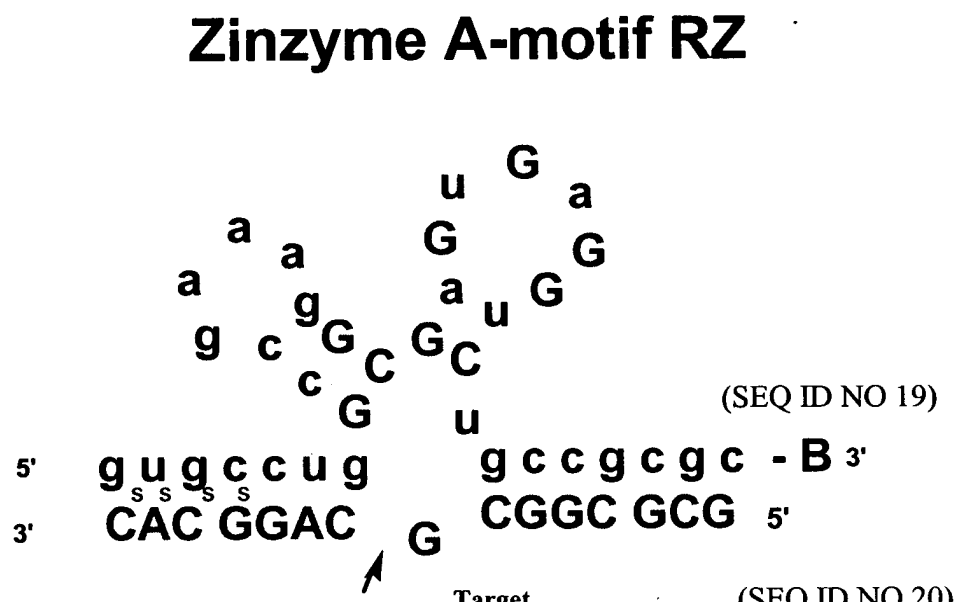
Figure 2: 2'-*O*-Me substituted Amberzyme Enzymatic Nucleic Acid Motif



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Figure 3: Stabilized Zinzyme Ribozyme Motif



(SEQ ID NO 19)

(SEQ ID NO 20)

Legend

Uppercase indicates natural ribo residues

C indicates 2' - d-NH_2-C

Lowercase: 2'-*O*- Me

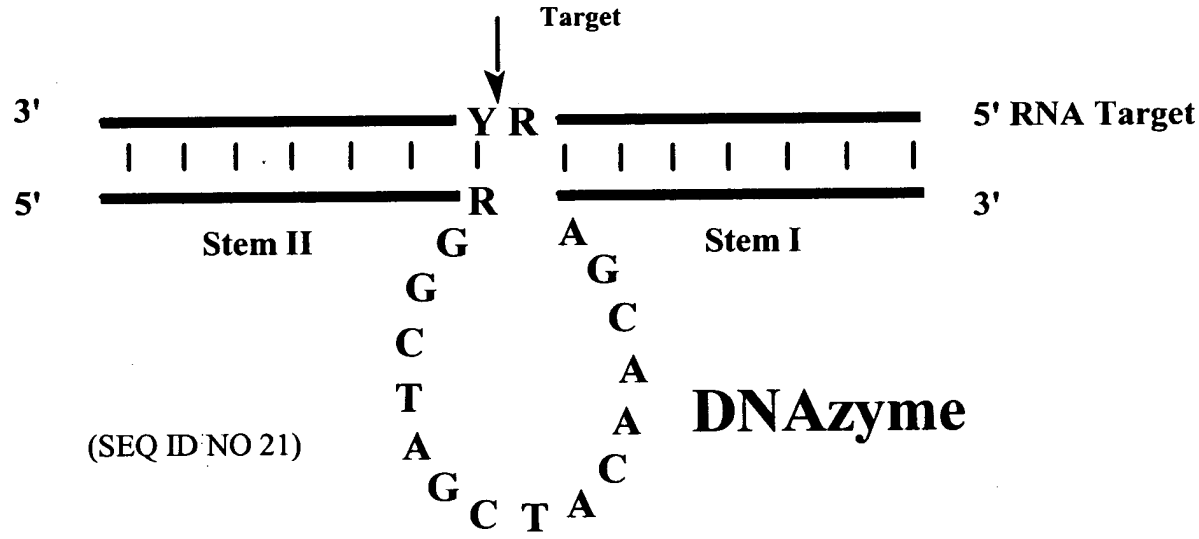
Subscript $_S$ indicates phosphothioate linkage

B: 3'-3' abasic moiety

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Figure 4: DNAzyme Motif

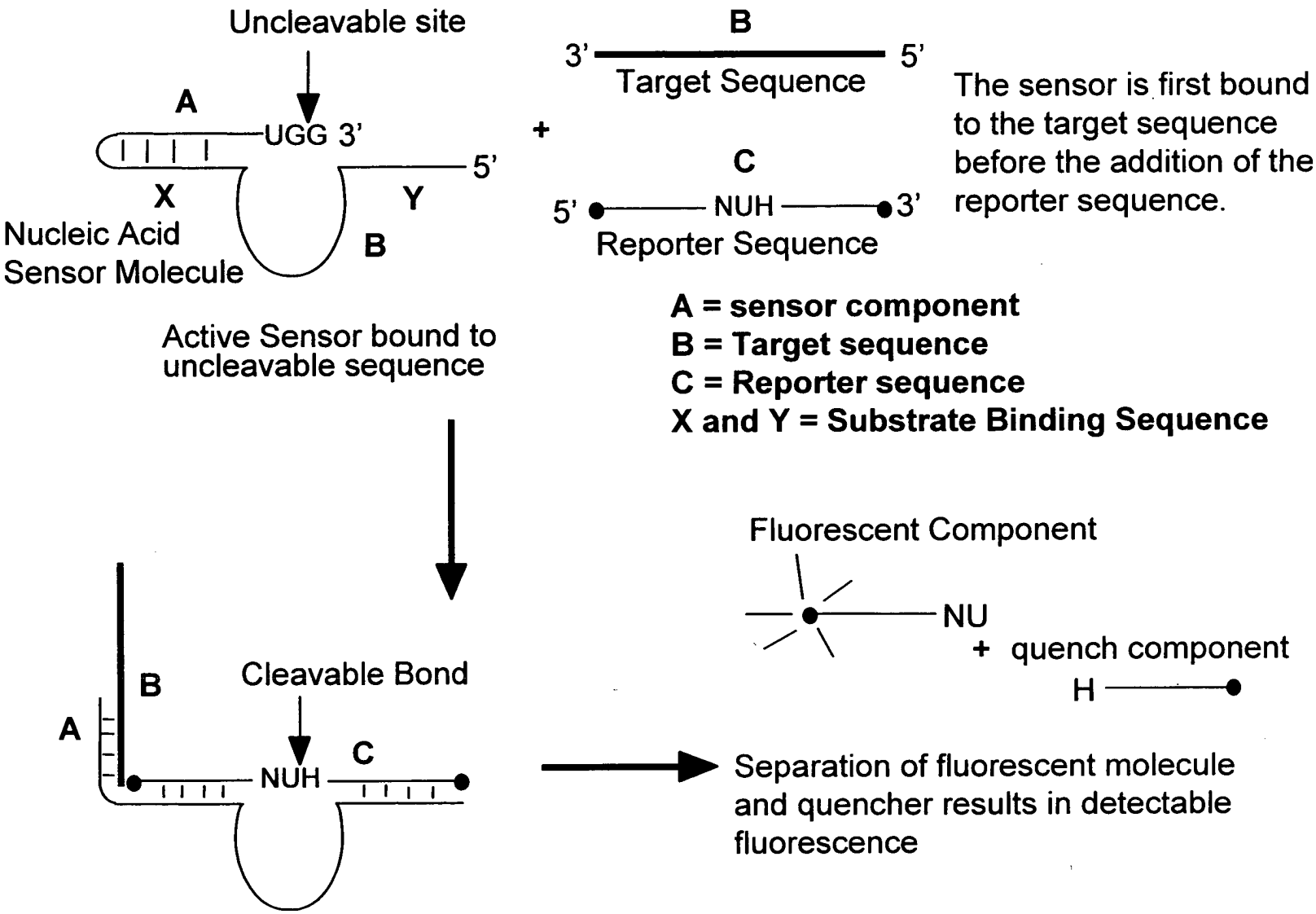


Legend

Y = U or C
R = A or G

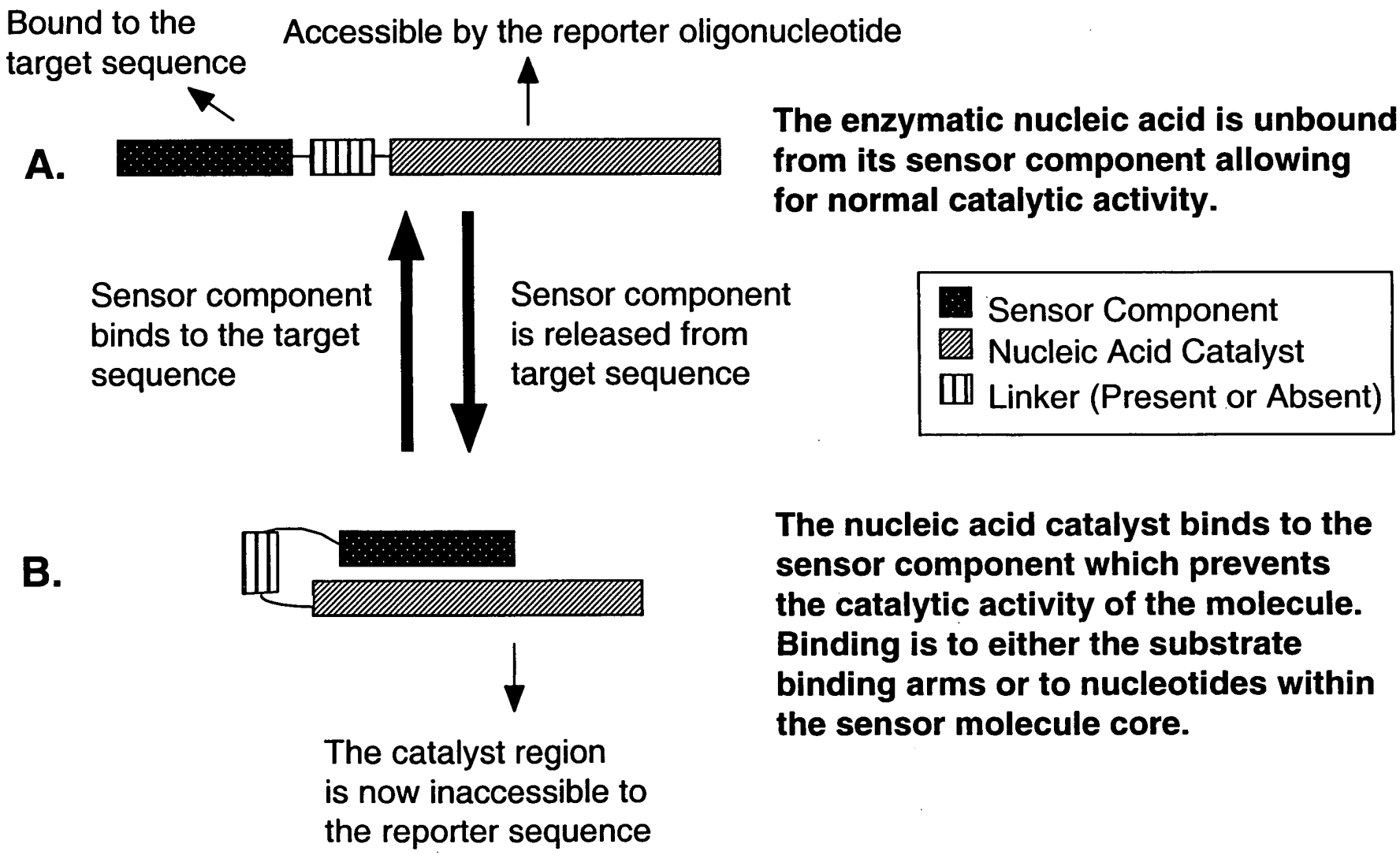
20020"T9795007

Figure 5. Detection of Target Sequence Using a Cis-Blocking Sequence



20E2T0"T9Z9500T

Figure 6. Schematic Diagram Representing the Two Primary Configurations of the Diagnostic effector molecule

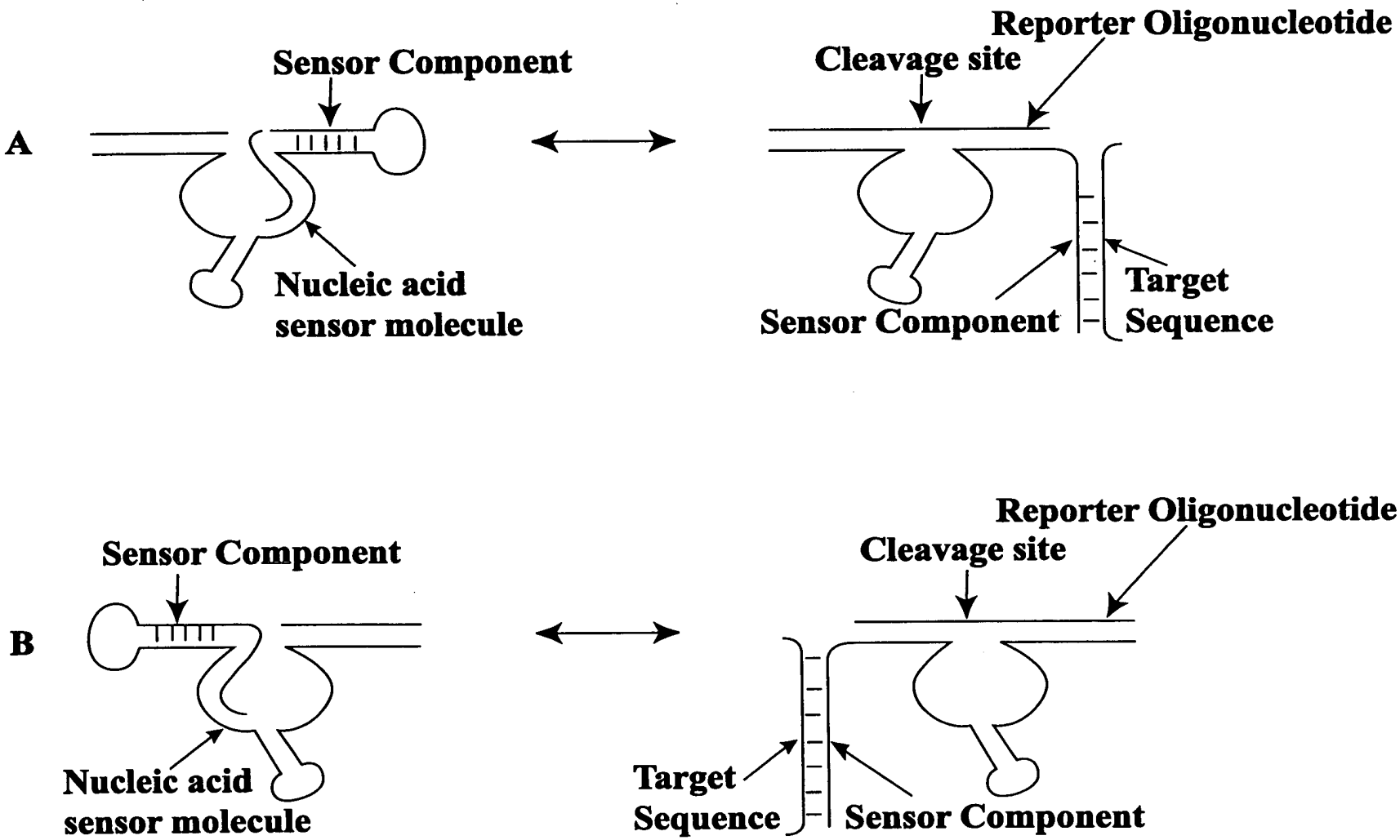


The enzymatic nucleic acid is unbound from its sensor component allowing for normal catalytic activity.

The nucleic acid catalyst binds to the sensor component which prevents the catalytic activity of the molecule. Binding is to either the substrate binding arms or to nucleotides within the sensor molecule core.

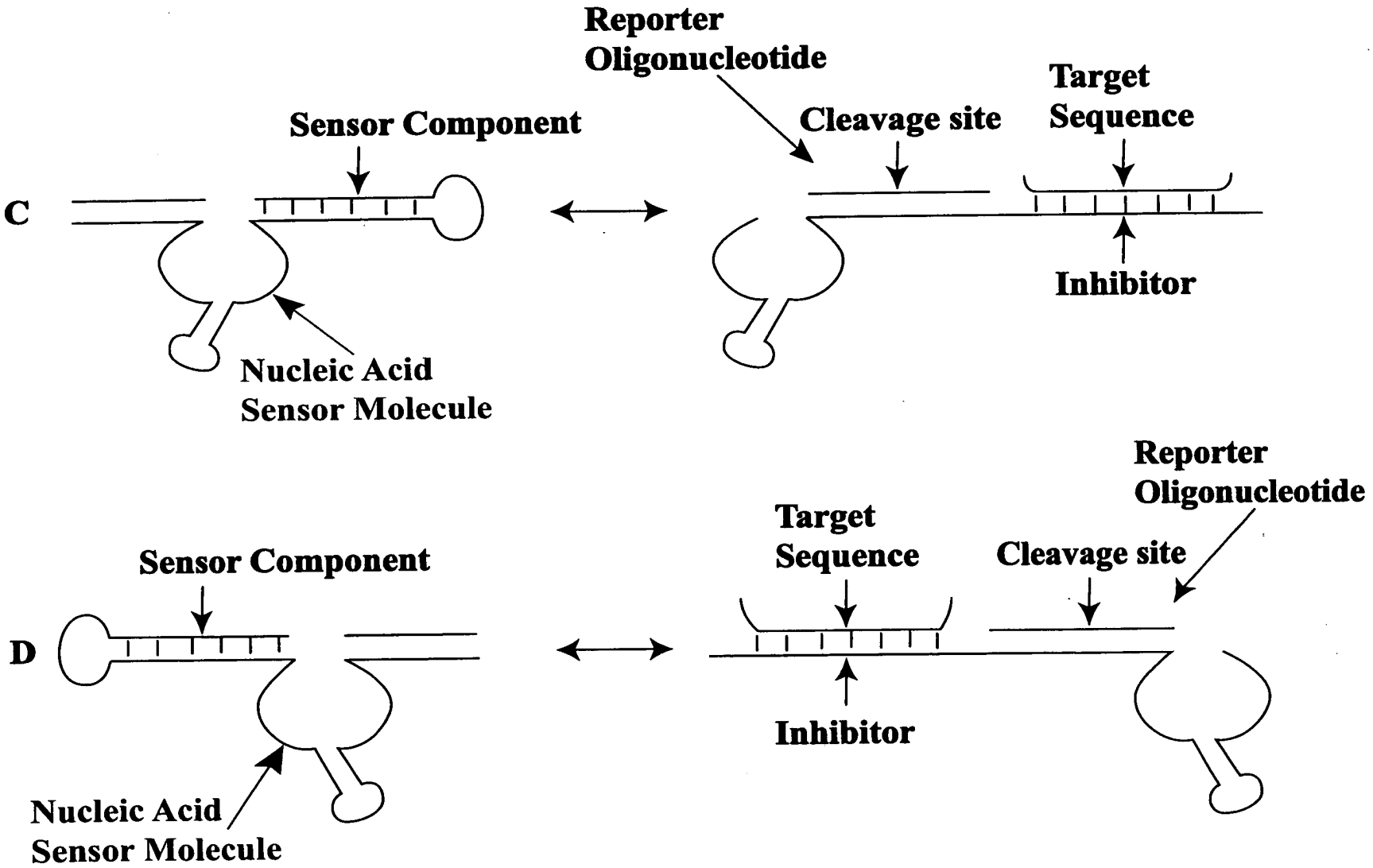
20220 T9795007

Figure 7a. Examples of Diagnostic Effector Molecules



20E2T0" T9Z9500T

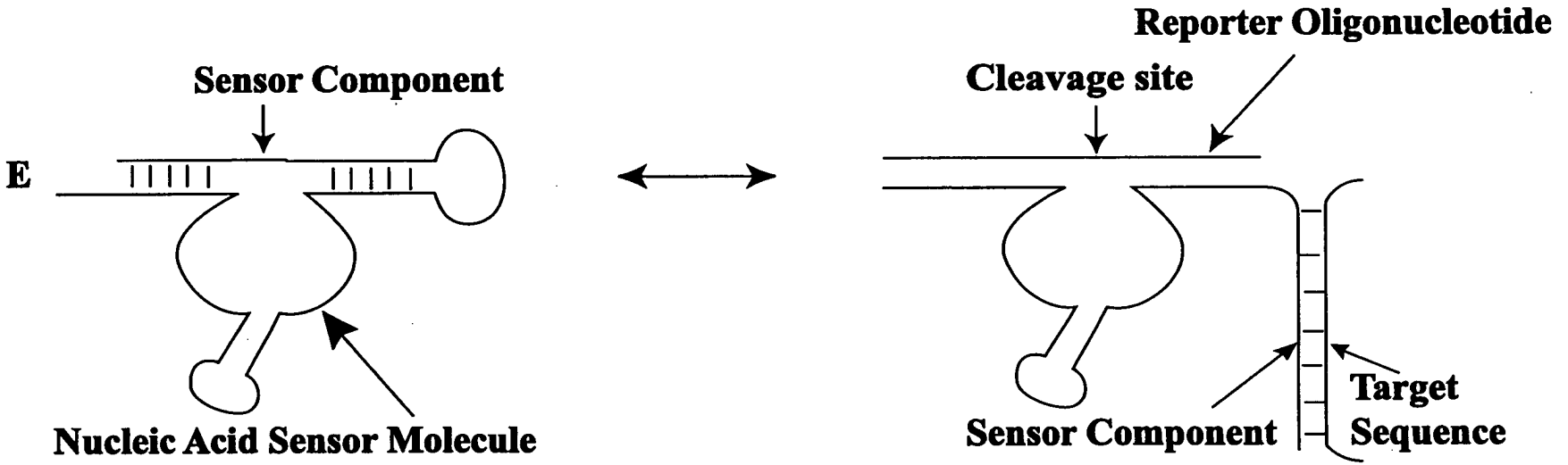
Figure 7b. Examples of Diagnostic Effector Molecules

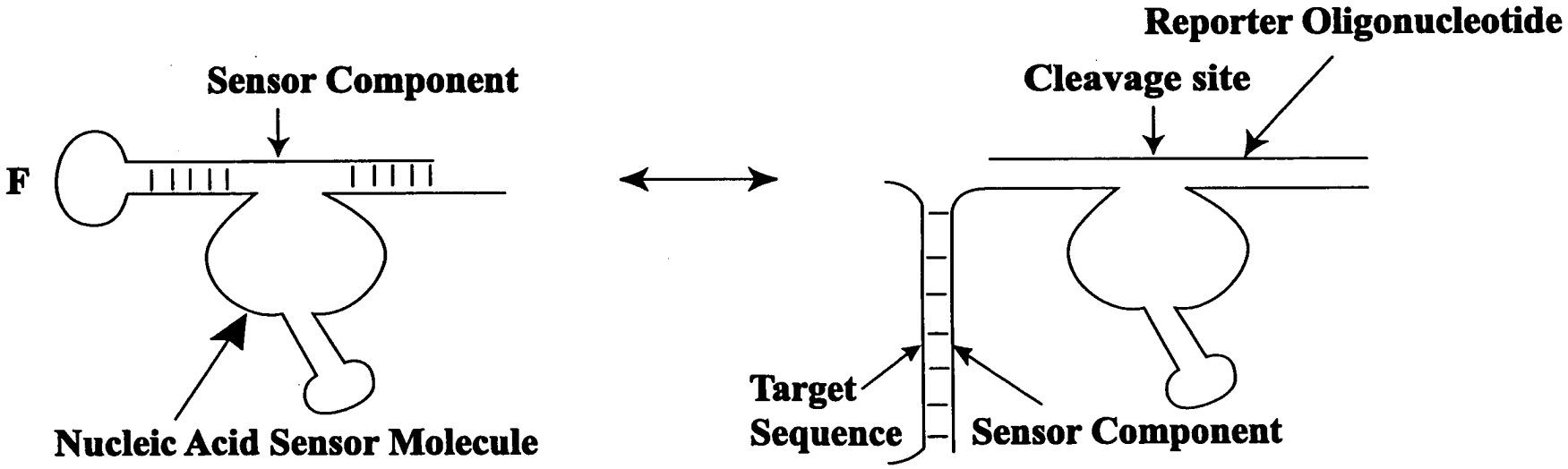


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20E270 "T9795007

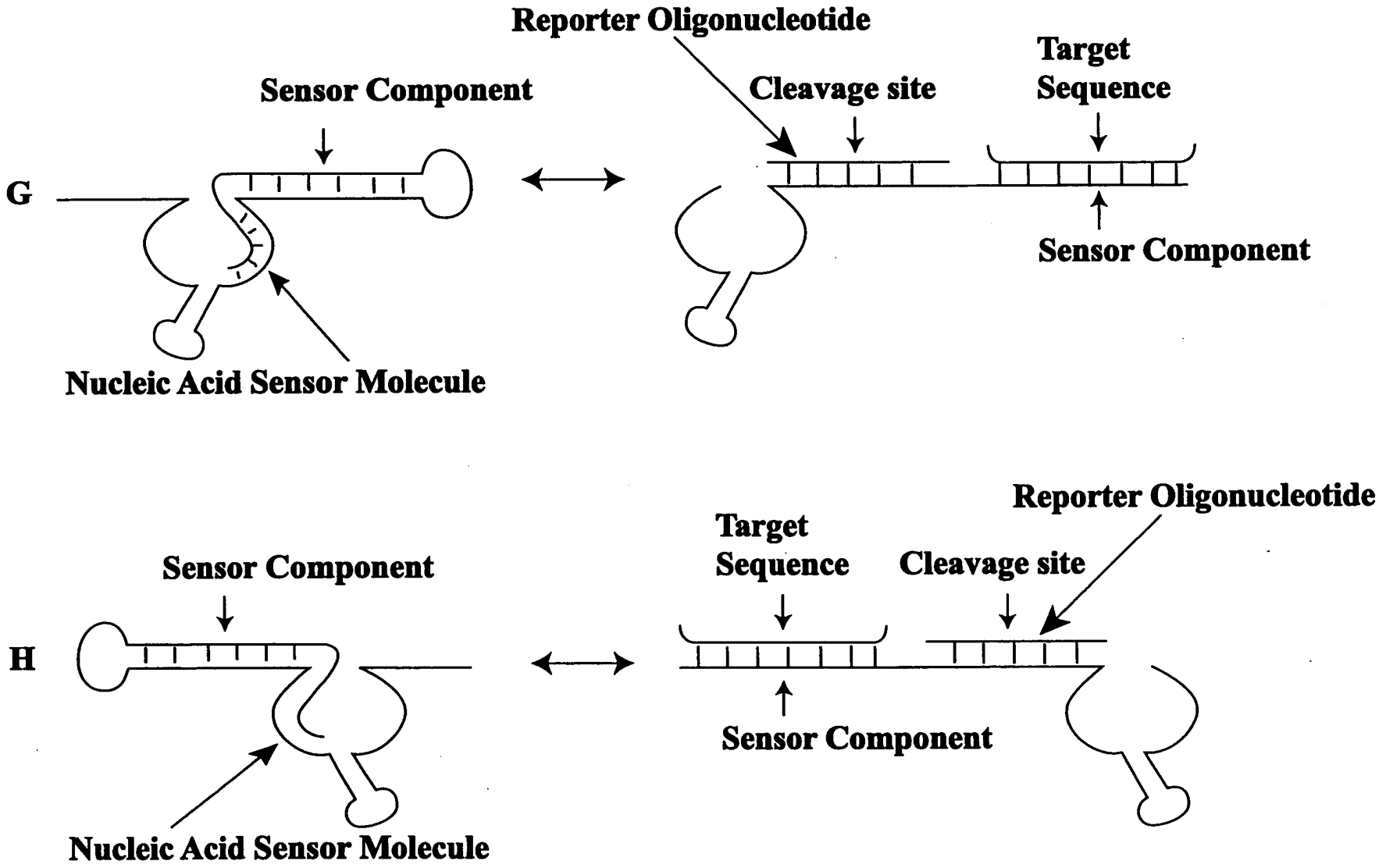
Figure 8a. Examples of Diagnostic Effector Molecules





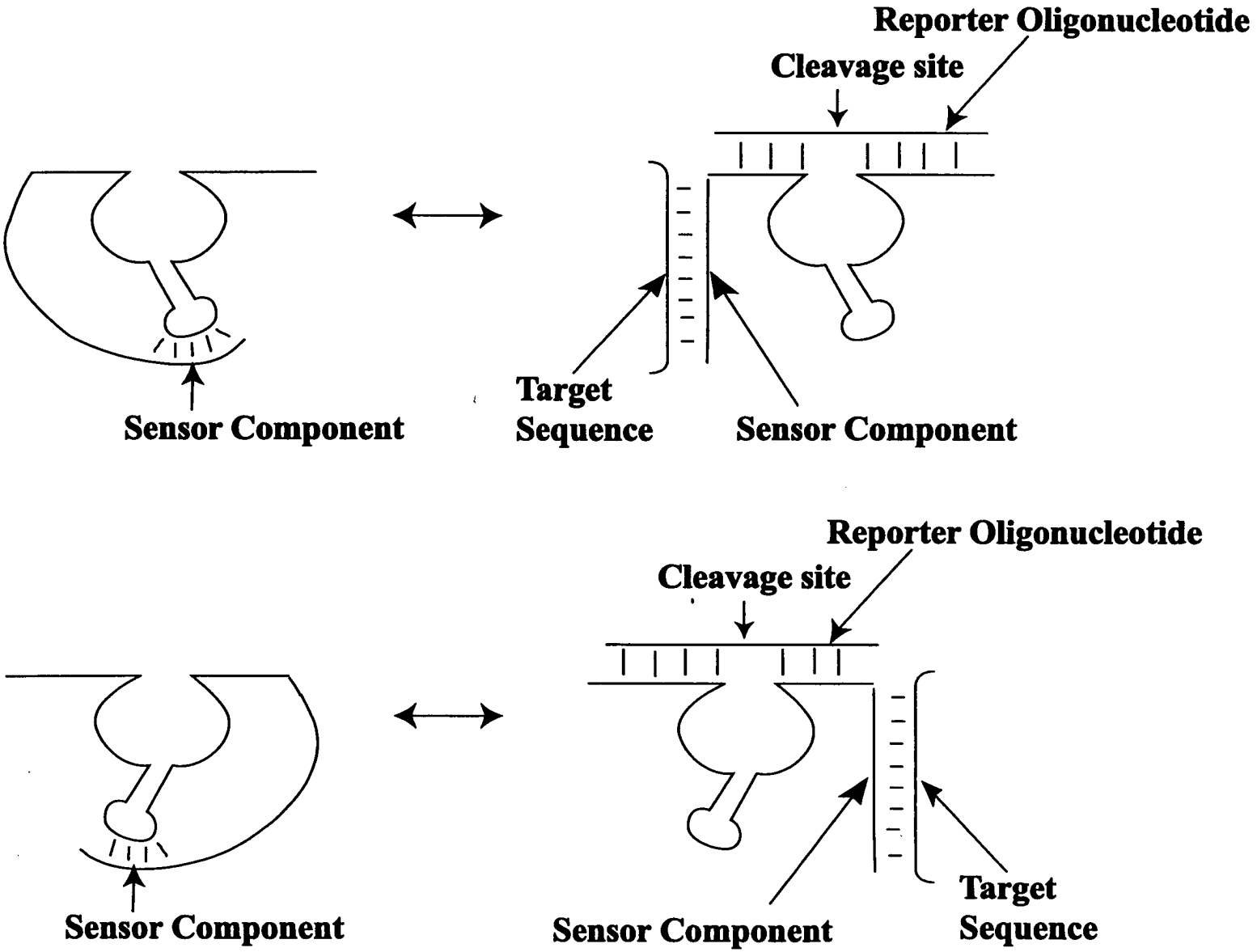
20E2T0" T9Z9500T

Figure 8b. Examples of Diagnostic Effector Molecules



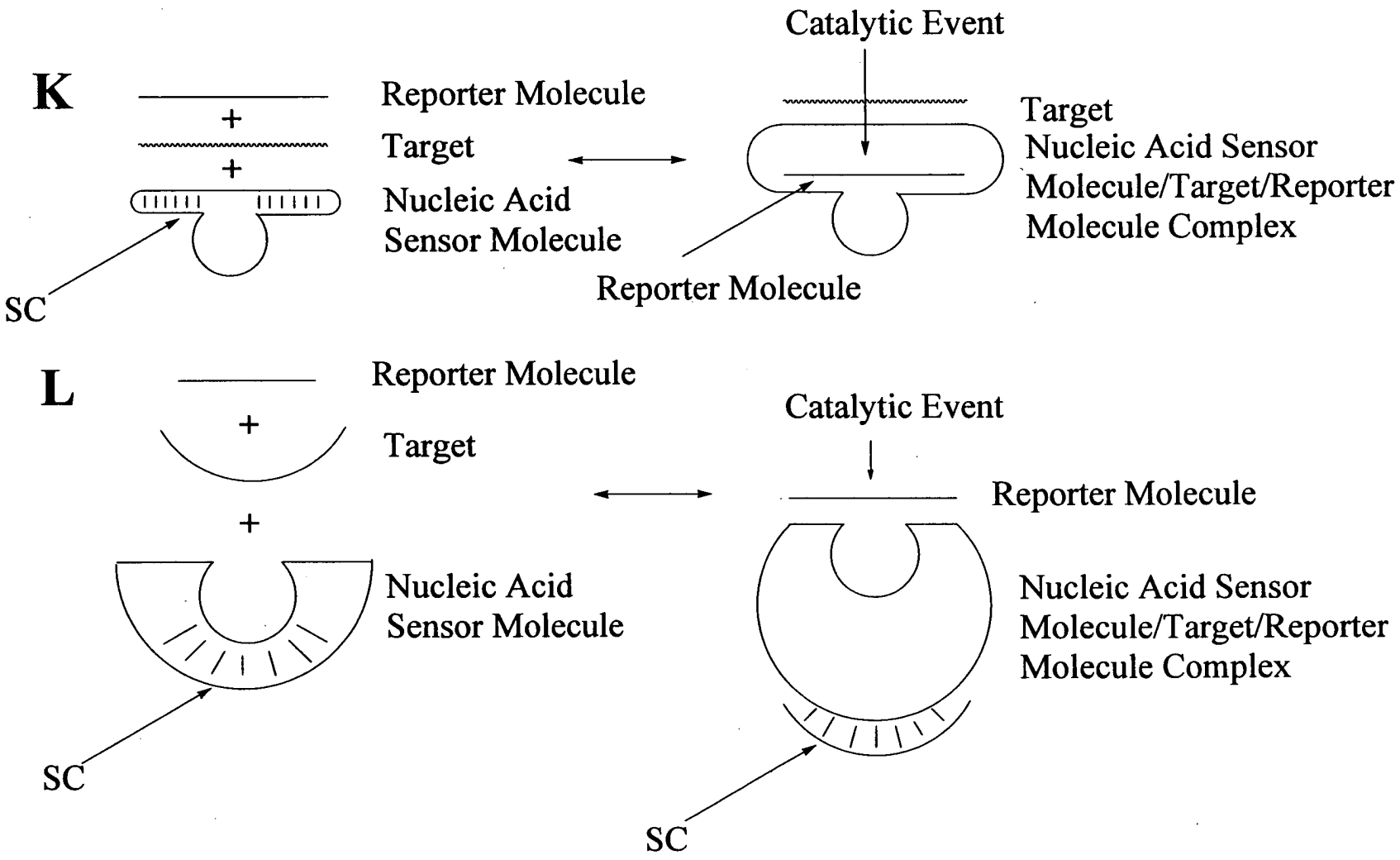
20E2TO°T9Z9500T

Figure 9. Examples of Diagnostic Effector Molecules



20E2T0" T9Z9500T

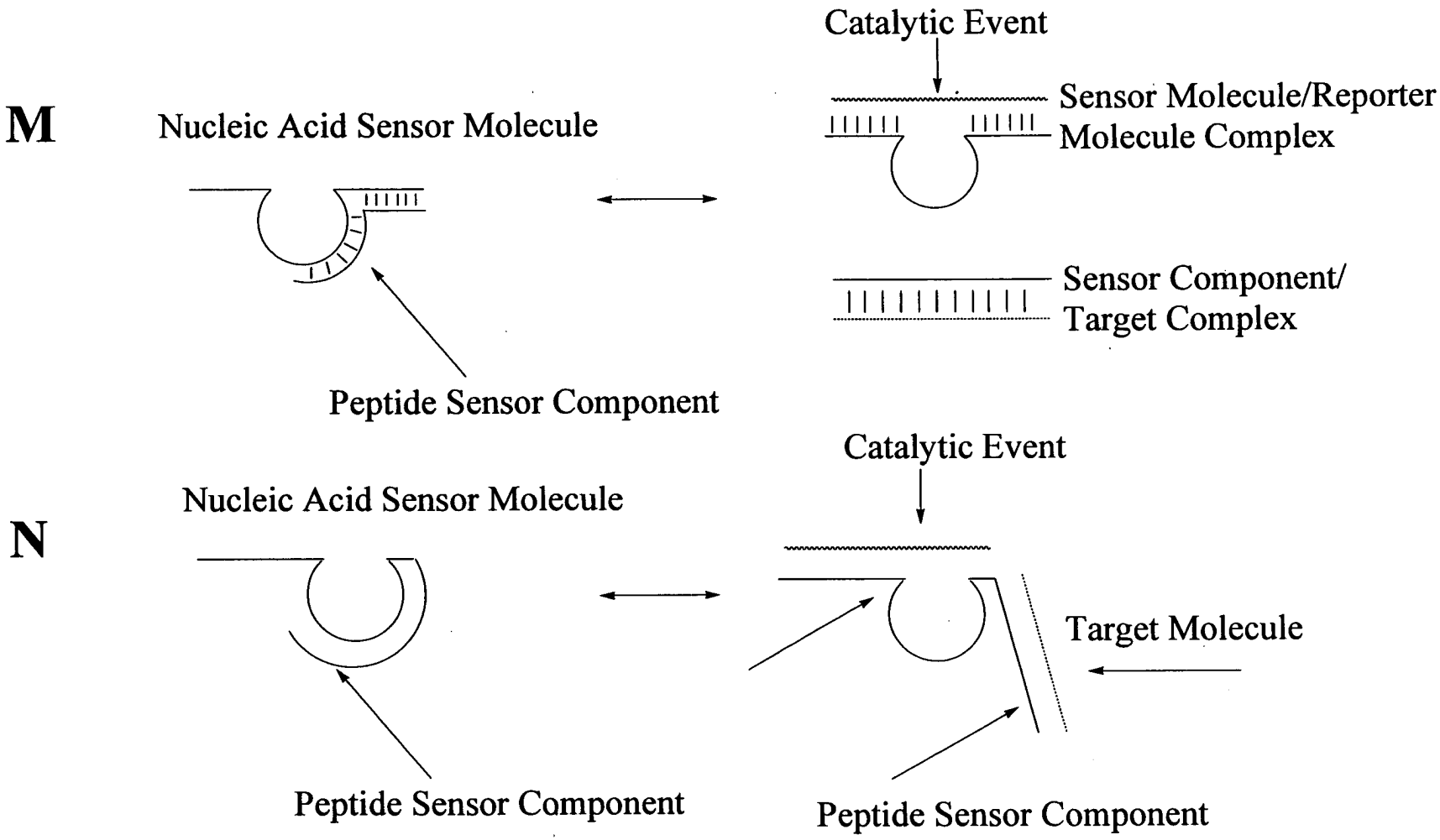
Figure 10: Examples of Diagnostic Effector Molecules



SC = Sensor Component

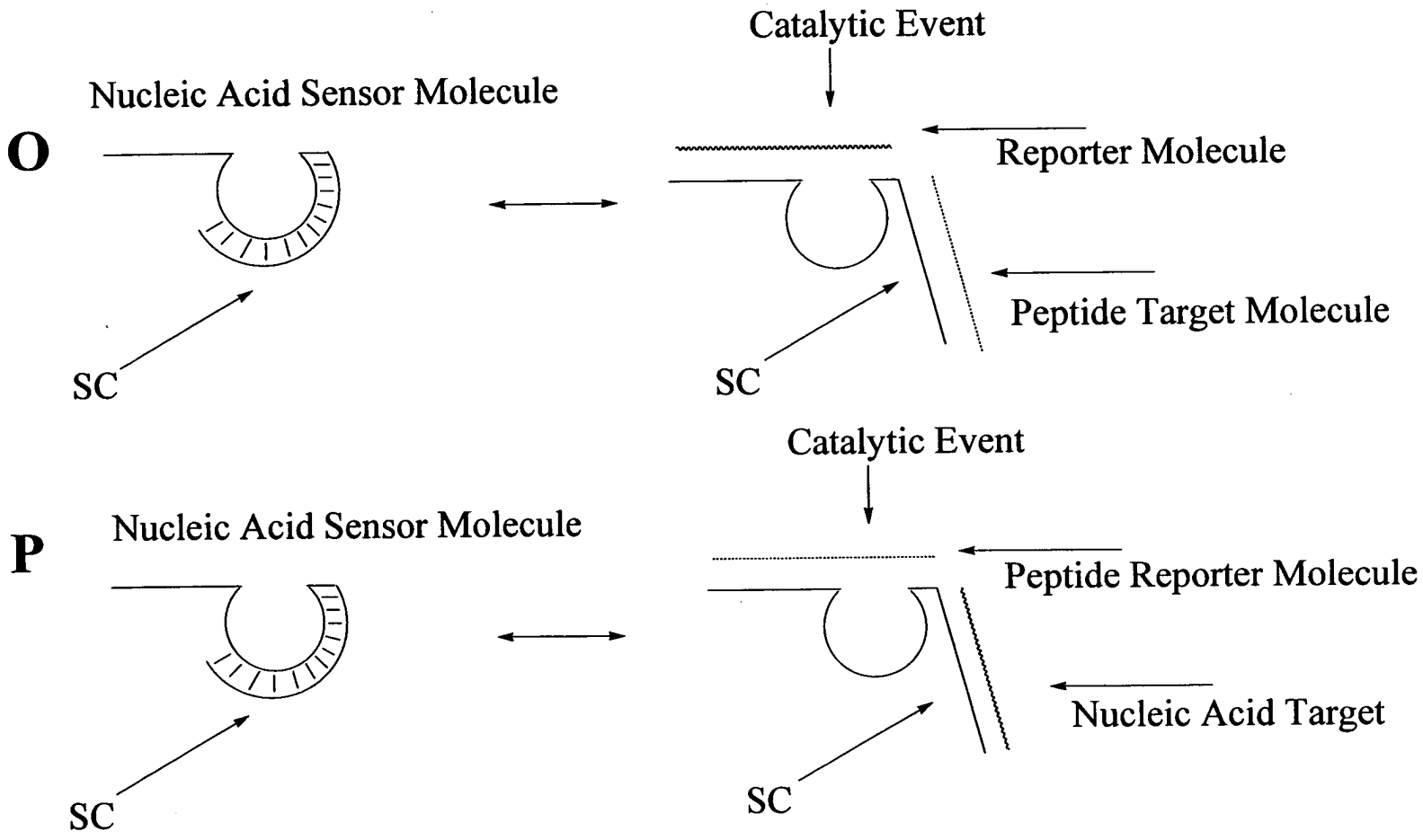
20220 T9795001

Figure 11: Examples of Diagnostic Effector Molecules



20E2T0"T9Z95001

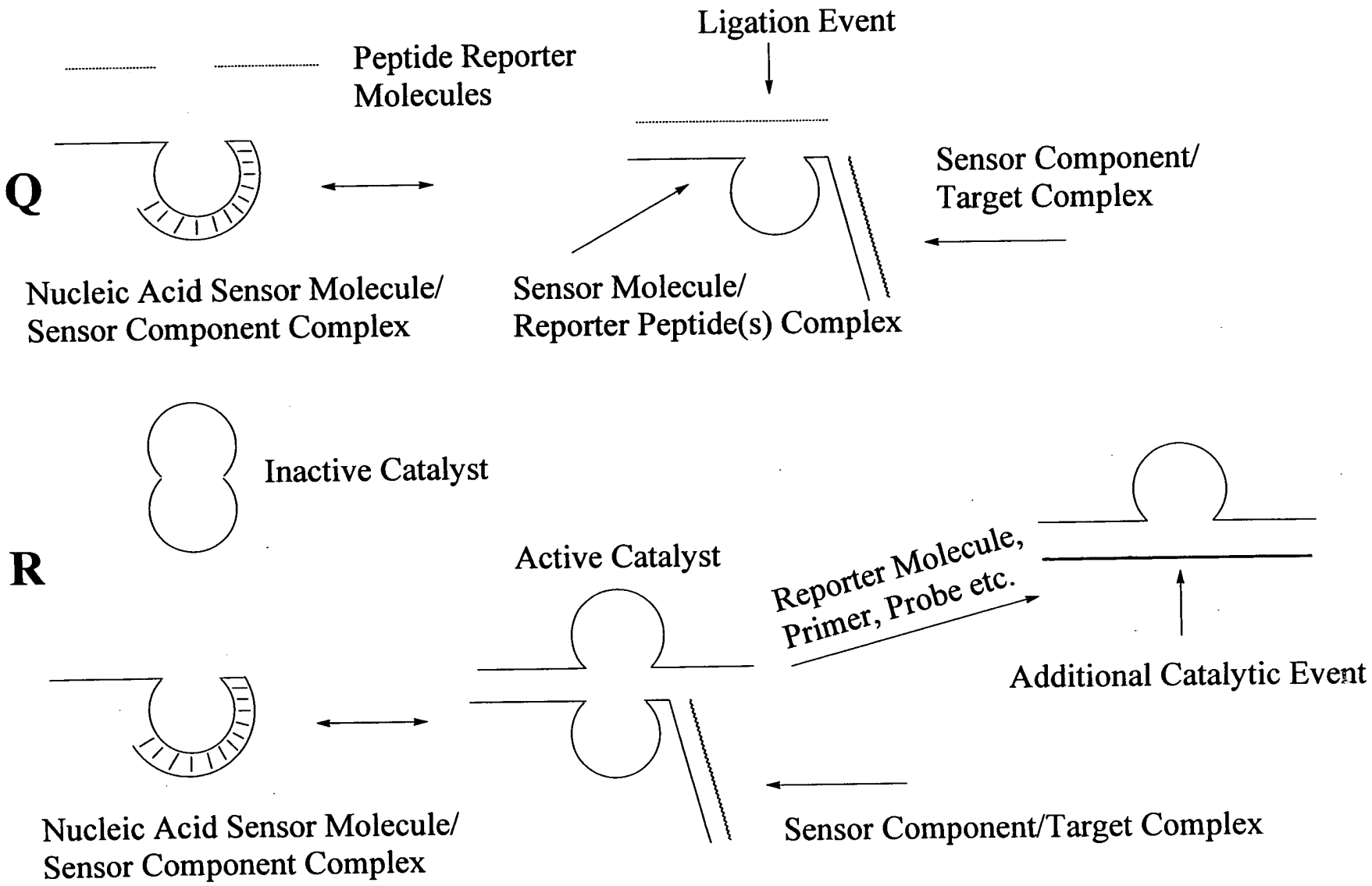
Figure 12: Examples of Diagnostic Effector Molecules



SC = Sensor Component

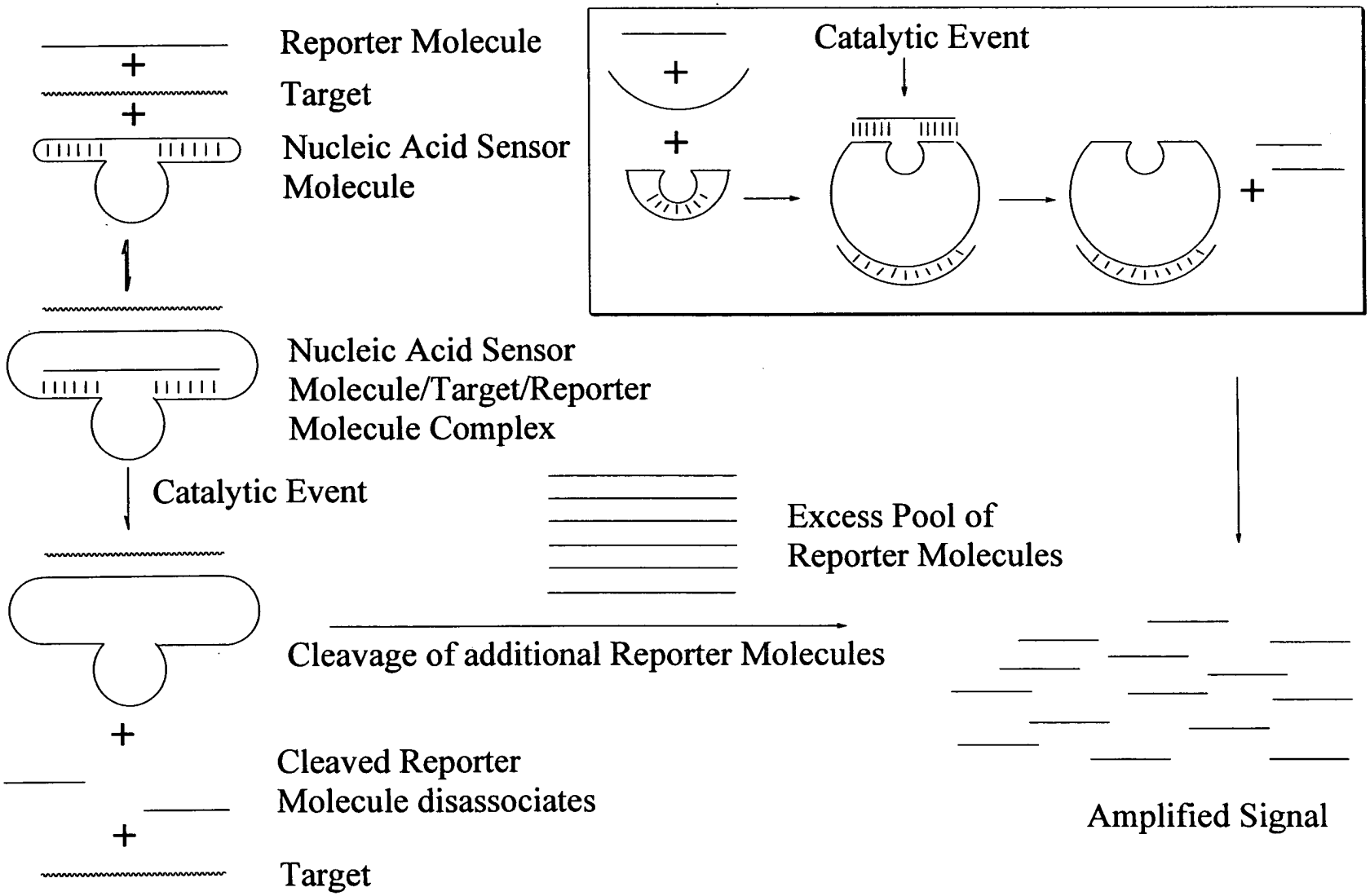
20E2T0" T9Z95001

Figure 13: Examples of Diagnostic Effector Molecules



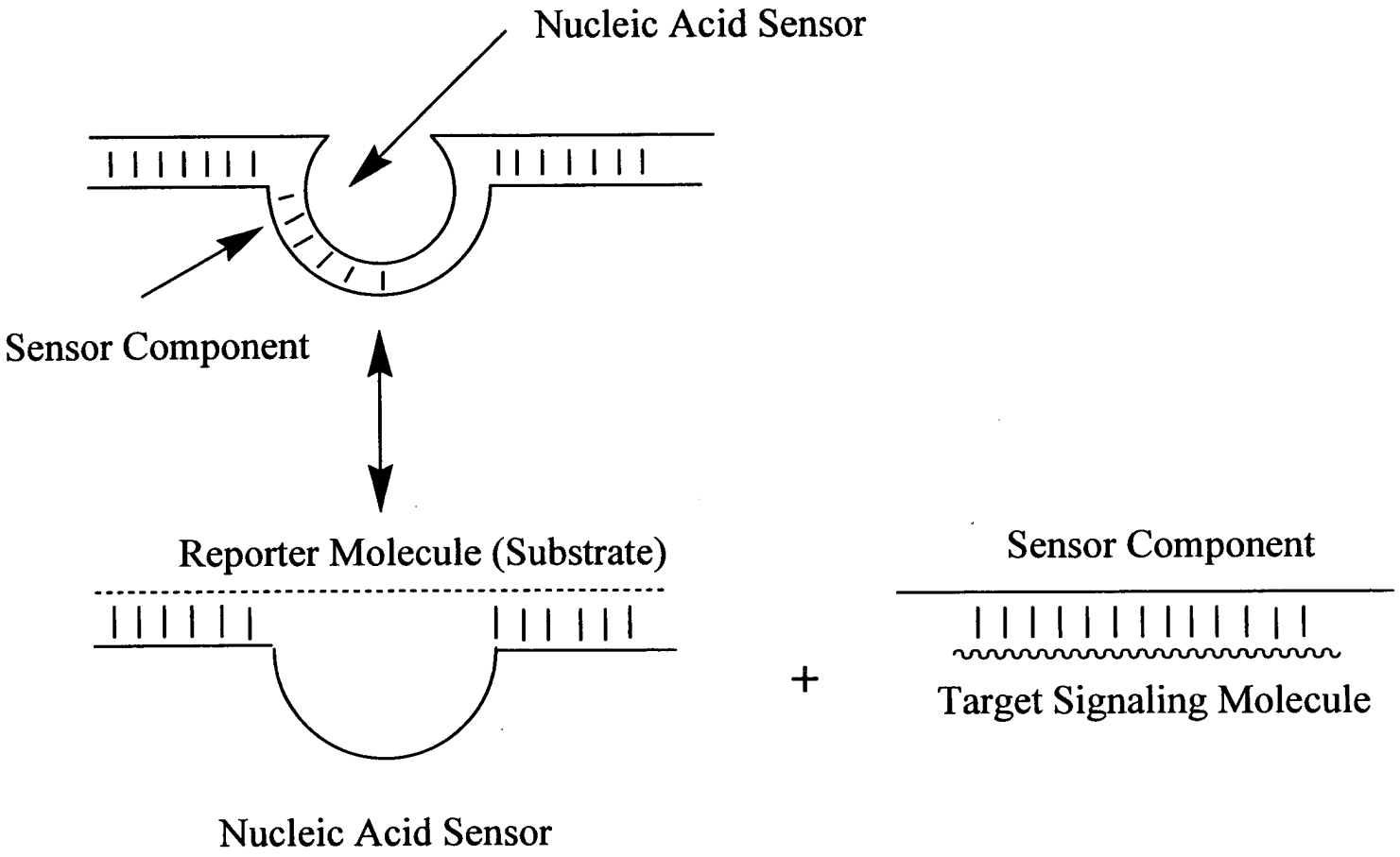
20020"T9795007

Figure 14: Inherent Amplification of Signal



20020°T9295001

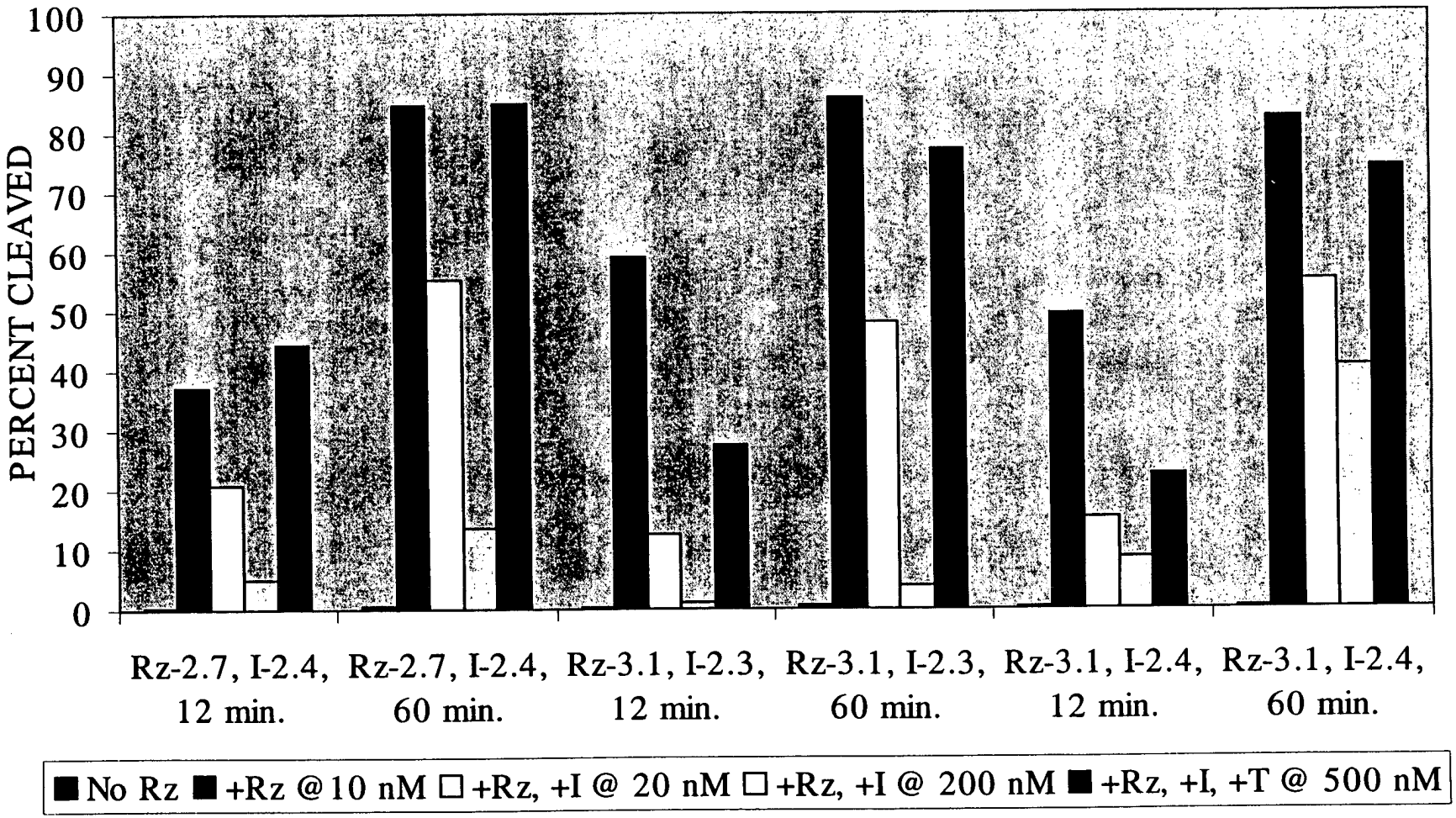
Figure 15: Example of Diagnostic System



20E2T0" T9Z9S00T

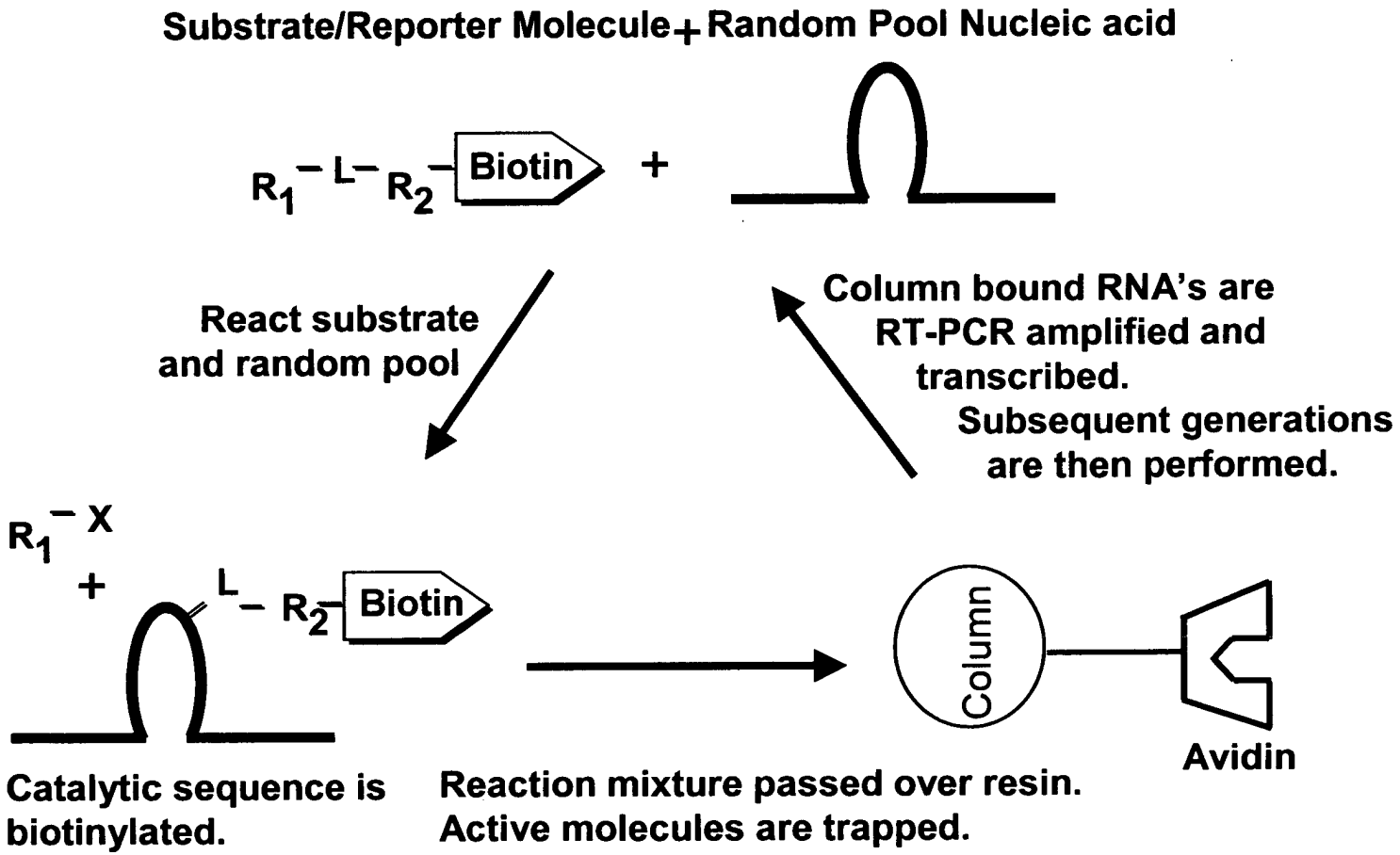
Figure 16: Ribozyme Diagnostic Screen

INHIBITORY FOLDING WITH TARGET RESCUE



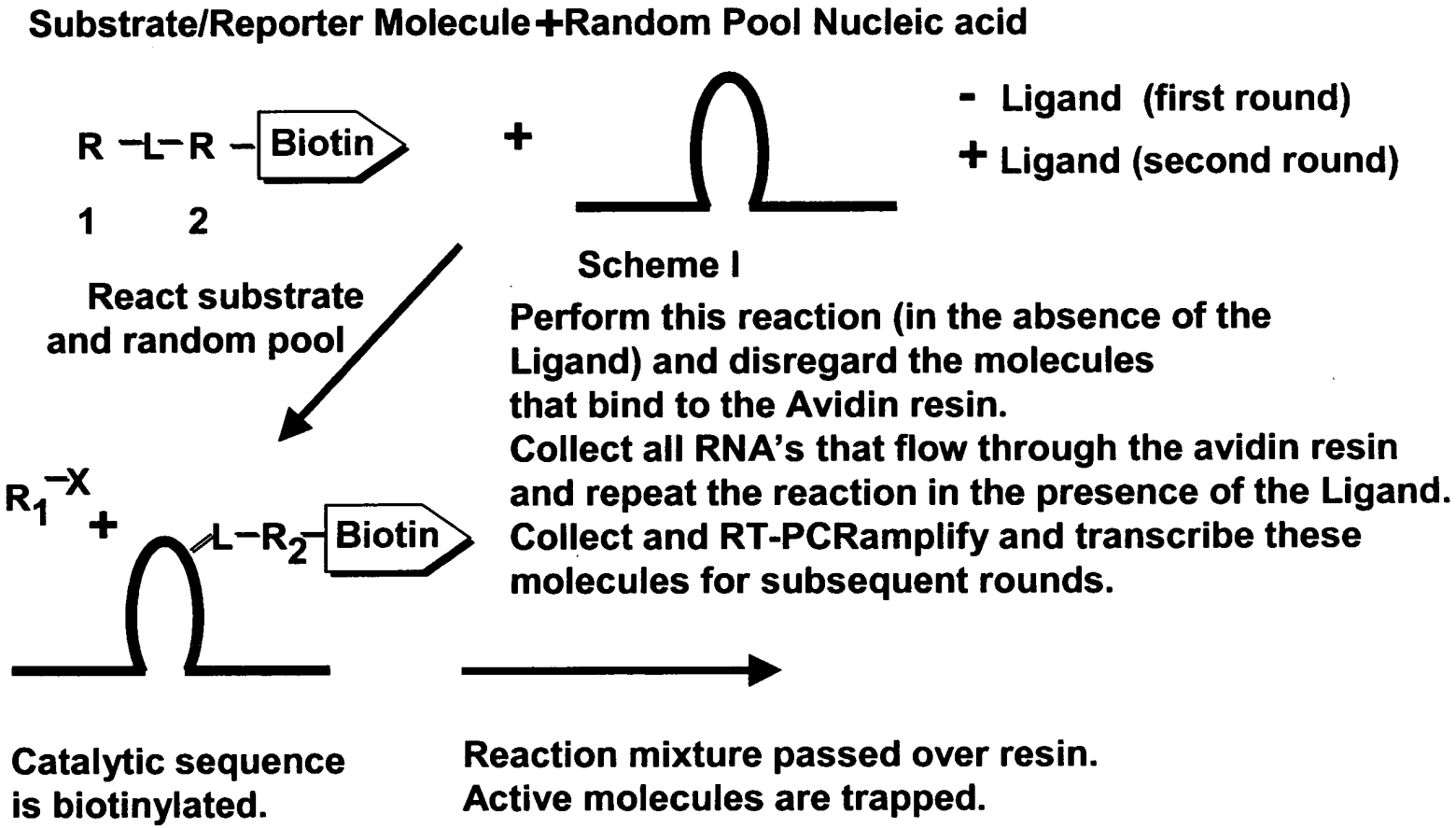
20E2TO" T9Z9500T

Figure 17a: Auto-ligation Nucleic Acid Sensor Molecules - Selection Scheme



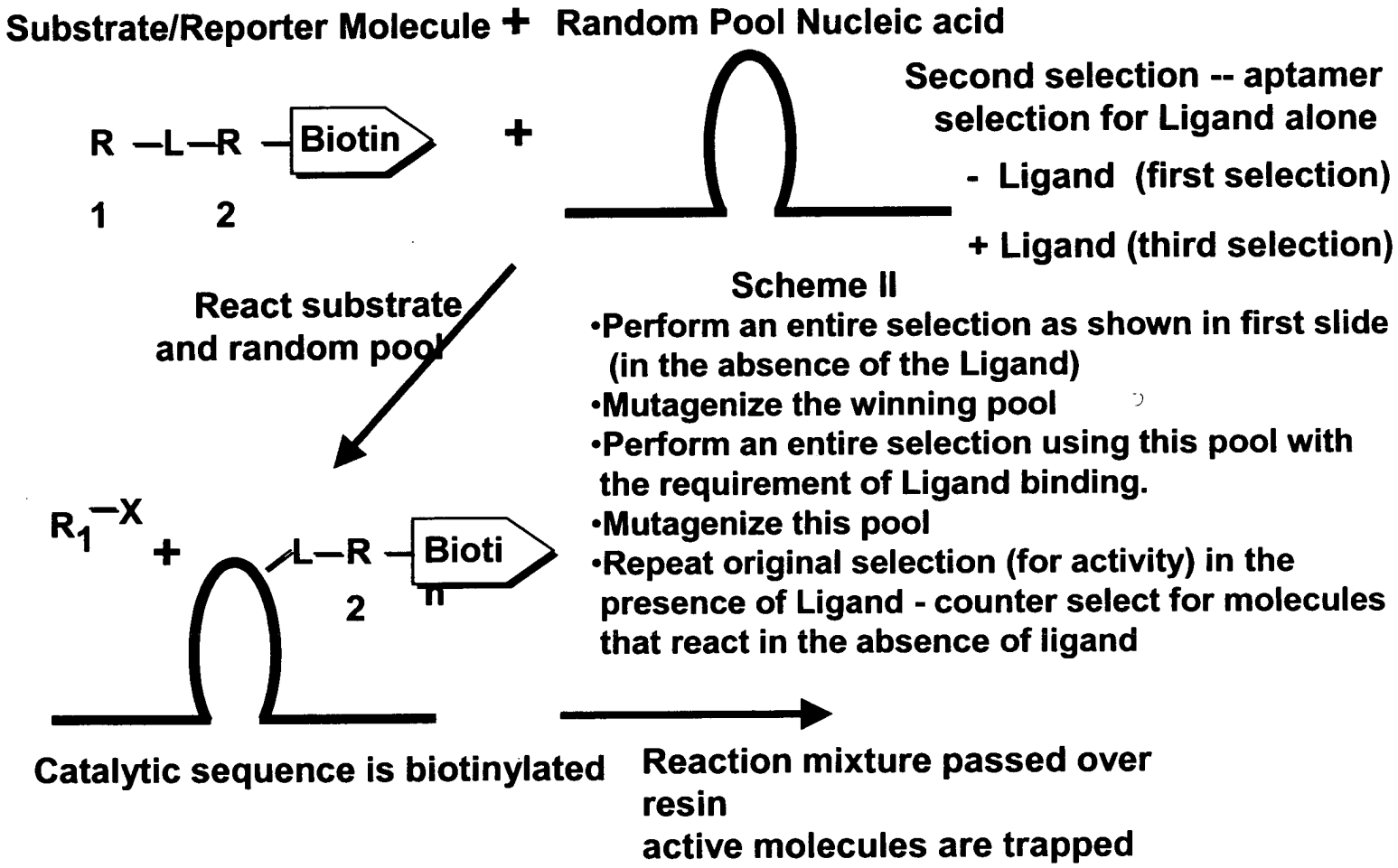
20E2T0" T9Z9S00T

Figure 17b: Auto-ligation Nucleic Acid Sensor Molecules - Ligand Dependent



20E2TO' T9Z9500T

Figure 17c: Auto-ligation Nucleic Acid Sensor Molecules- Ligand dependent



20E2T0 T9795001

Figure 18a: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme

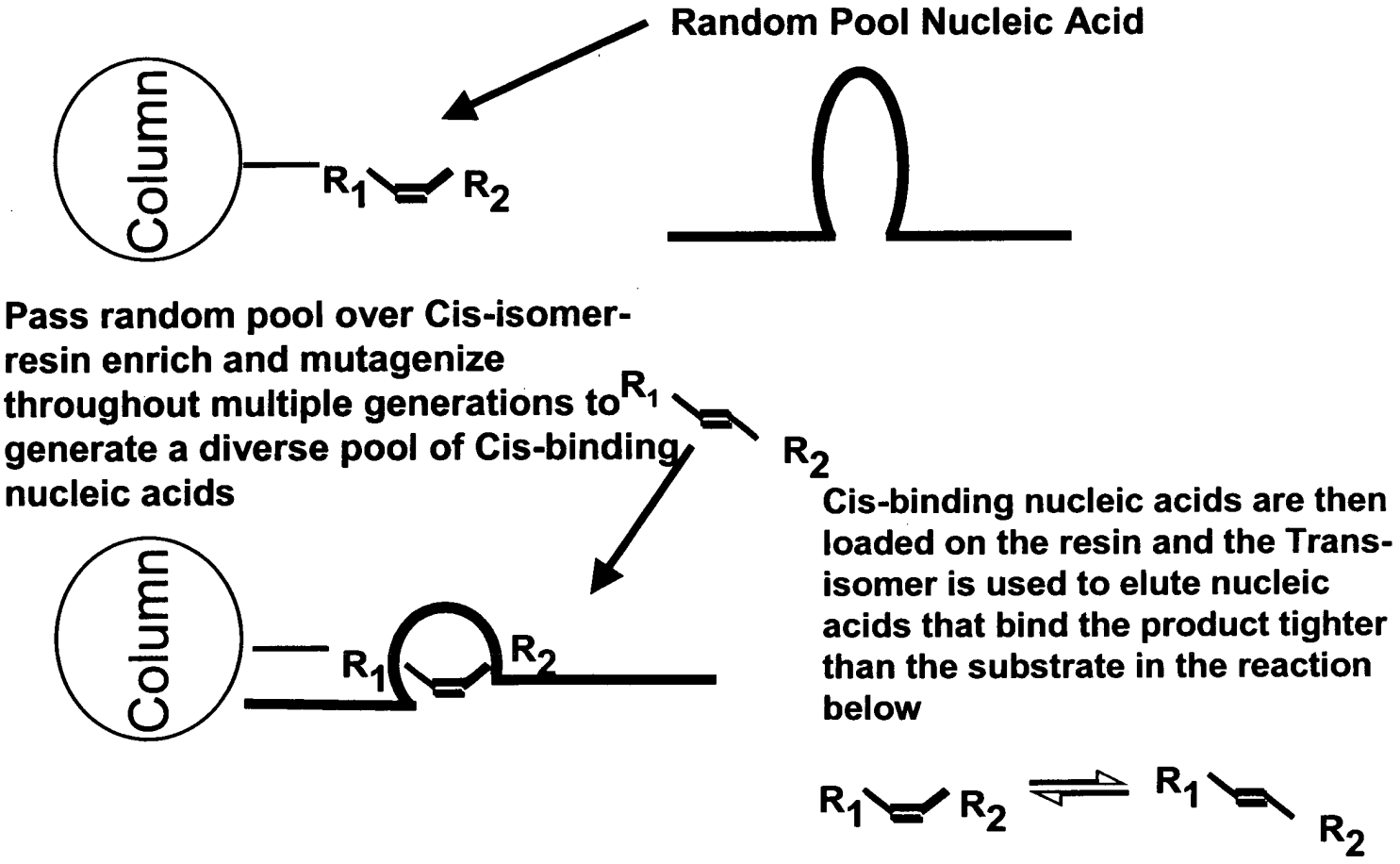
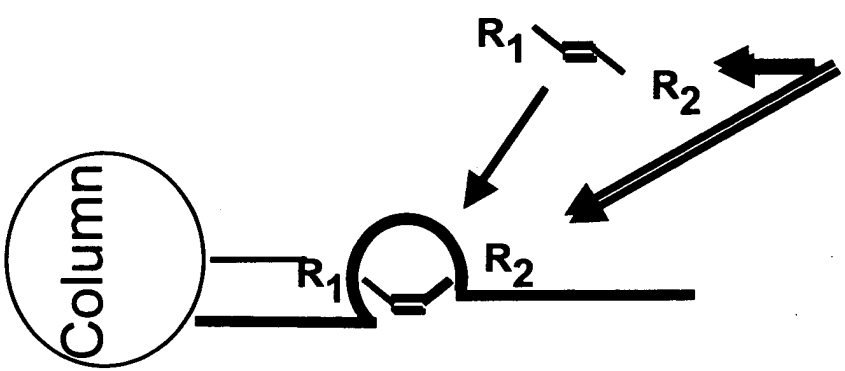


Figure 18b: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme



The concentration of ligand on the resin and the concentration of eluant ligand can be manipulated in order to bias for enzymatic nucleic acids that prefer one isomer over the other -and can therefore drive the reaction in a desired direction.

E.g. Selection for Cis-isomer at 100 uM - yeild $^{cis}K_d$ = 100 Um

Elute with Trans-isomer at 0.1 uM - yield $^{trans}K_d$ = 0.1 uM

Isolate catalysts for the reaction below



20E2TO" T9Z9500T

Figure 18c: Isomerase Nucleic Acid Sensor Molecule - Ligand dependent

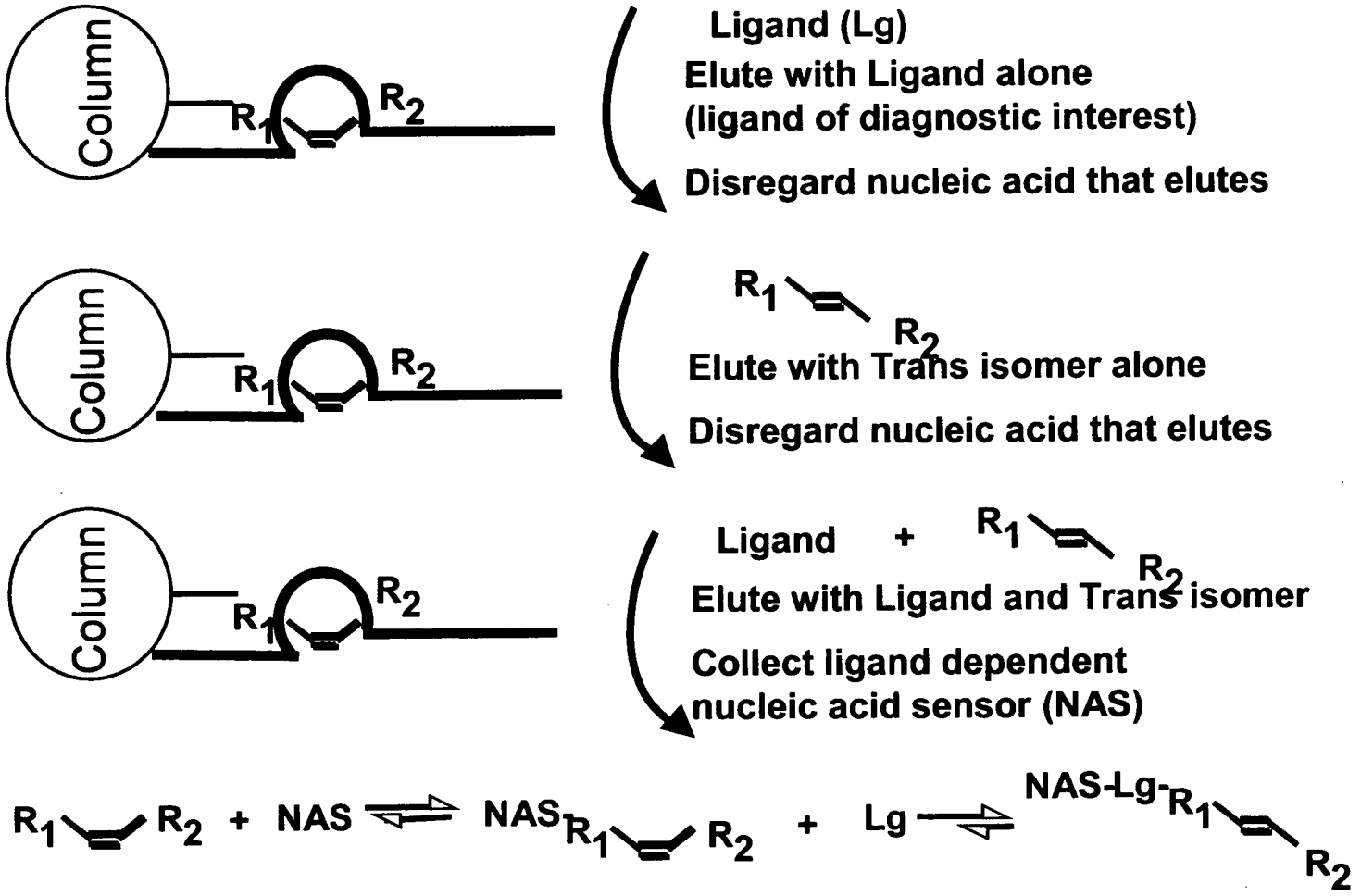
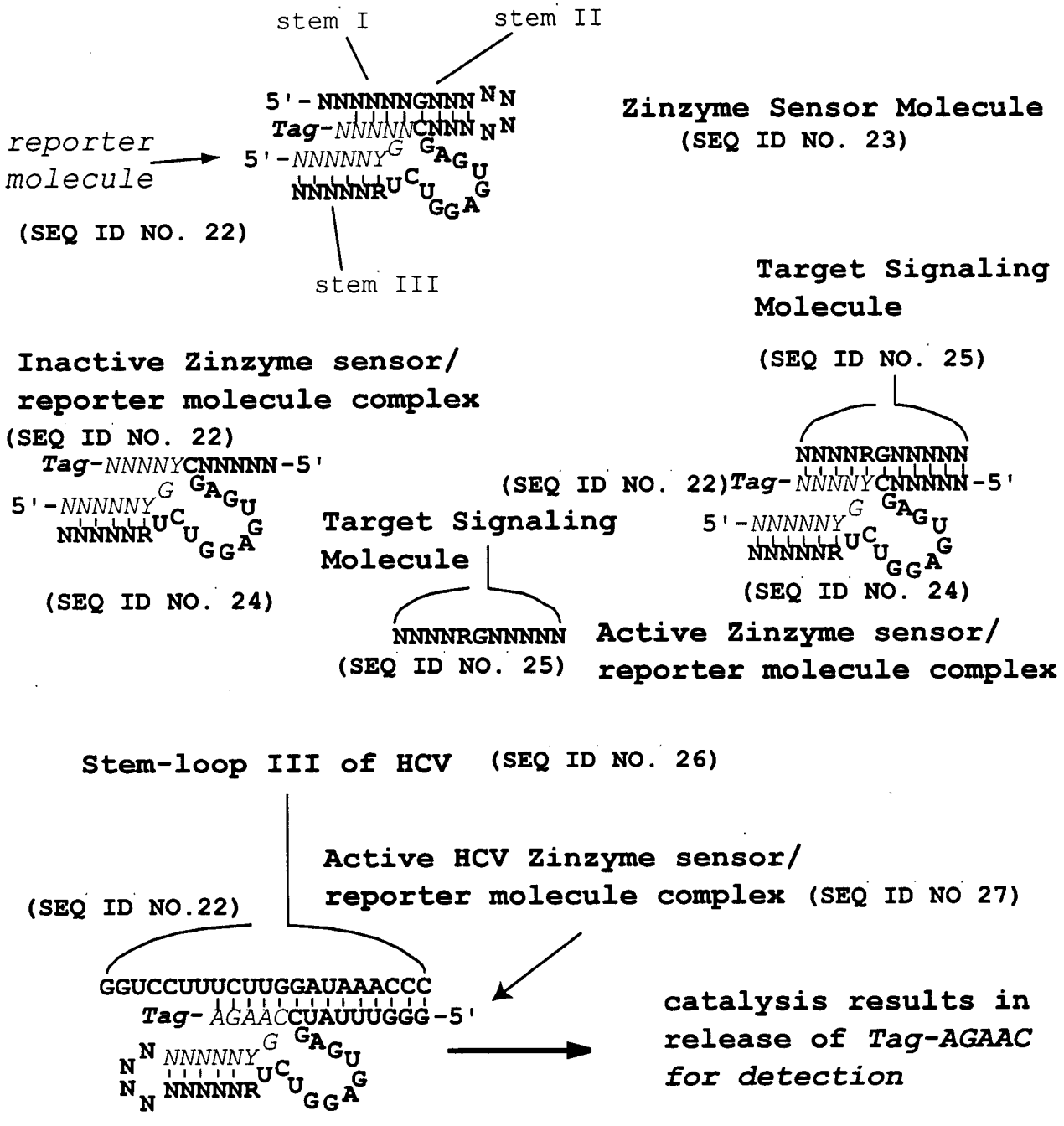


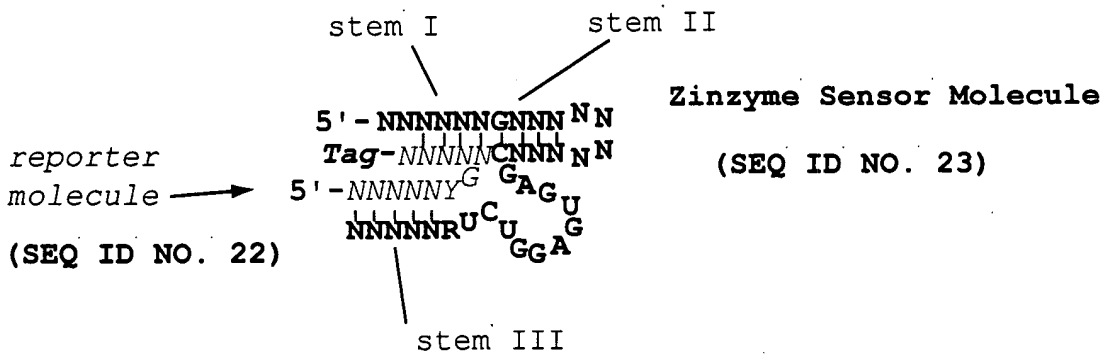
Figure 19: Zinzyme Sensor Molecule for detection of Nucleic Acid



Zinzyme sensor can be attached to solid support/surface, for example at the 5'-end

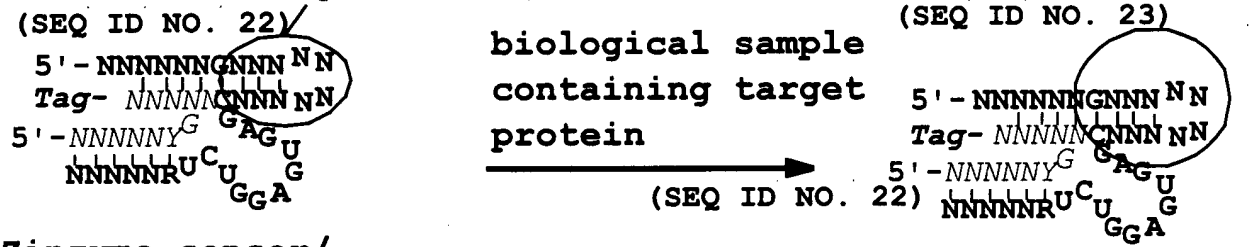
Docket: MBHB00-816-E

Figure 20: Zinzyme Sensor Molecule for detection of Protein



(SEQ ID NO. 23)
RNA sequence that binds a clinically relevant protein

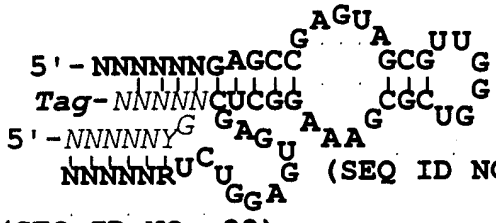
Protein interaction



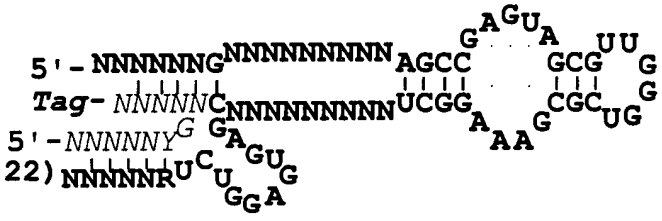
Zinzyme sensor/ reporter molecule complex

Activated/Inactivated Complex

Sensor/reporter complex for detection of HCV core protein



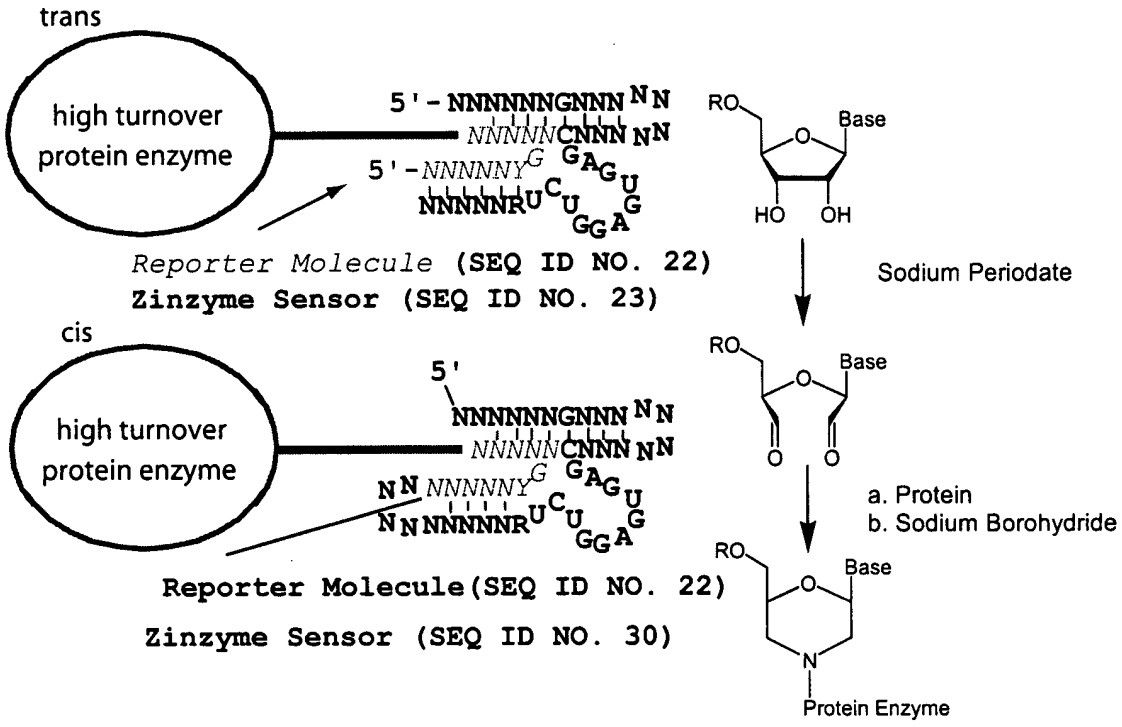
(SEQ ID NO. 22)
HCV Zinzyme sensor with loop IIID of HCV (directs the binding of HCV core protein)
(SEQ ID NO 28)



HCV Zinzyme sensor with loop IIID of HCV connected via randomized linker
(SEQ ID NO 29)

FOOSGNGT.TOOSGNGT

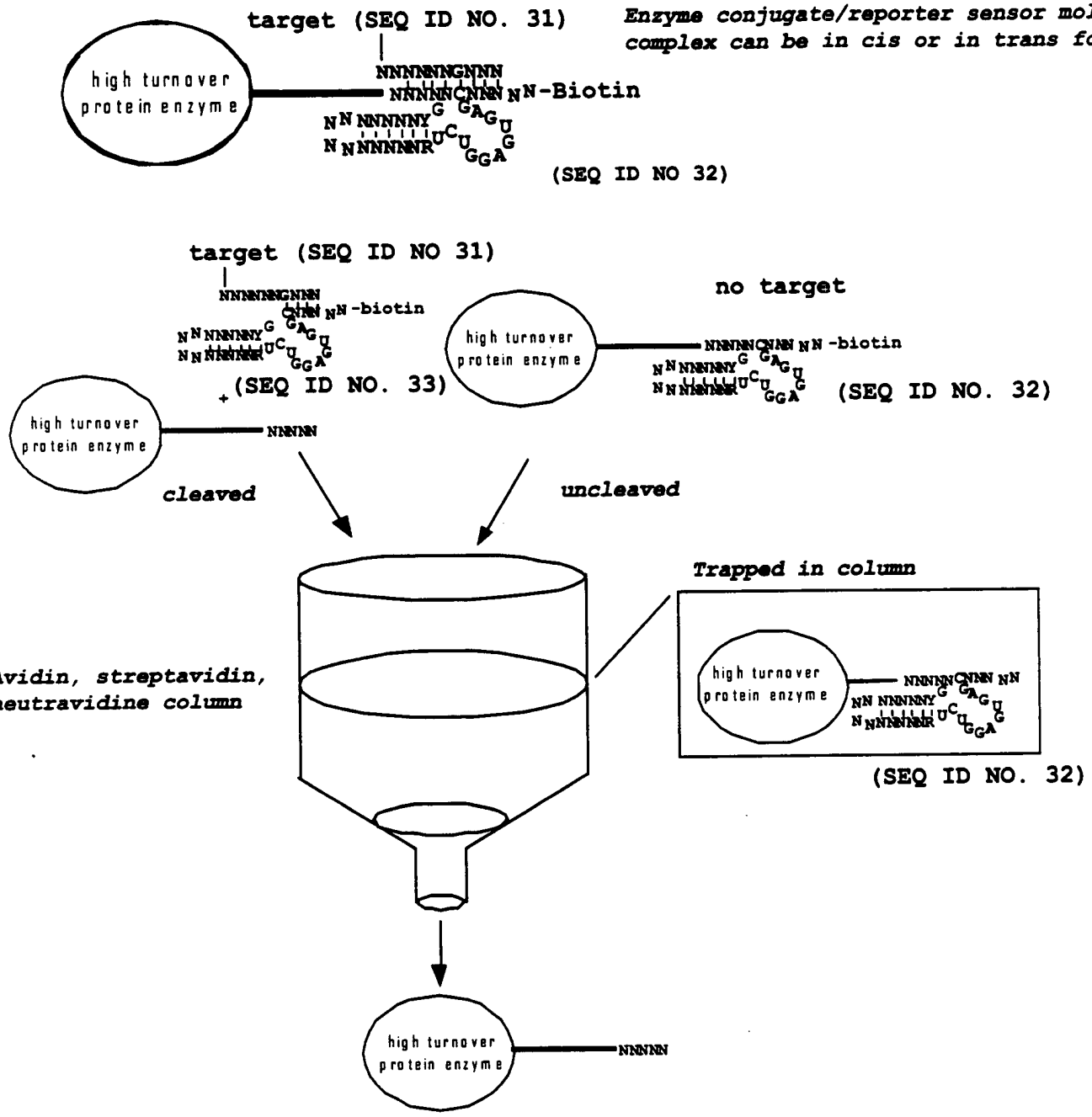
Figure 21: Zinzyme Sensor Molecule with protein enzyme reporter



R is oligonucleotide.
Protein can be attached via amino linker.
Alternately, R is phorphoramidite moiety for incorporation at 5'-end of oligonucleotide.

High turnover protein enzyme is, for example, Luciferase, Horseradish peroxidase, beta-galactosidase, alkaline phosphatase.

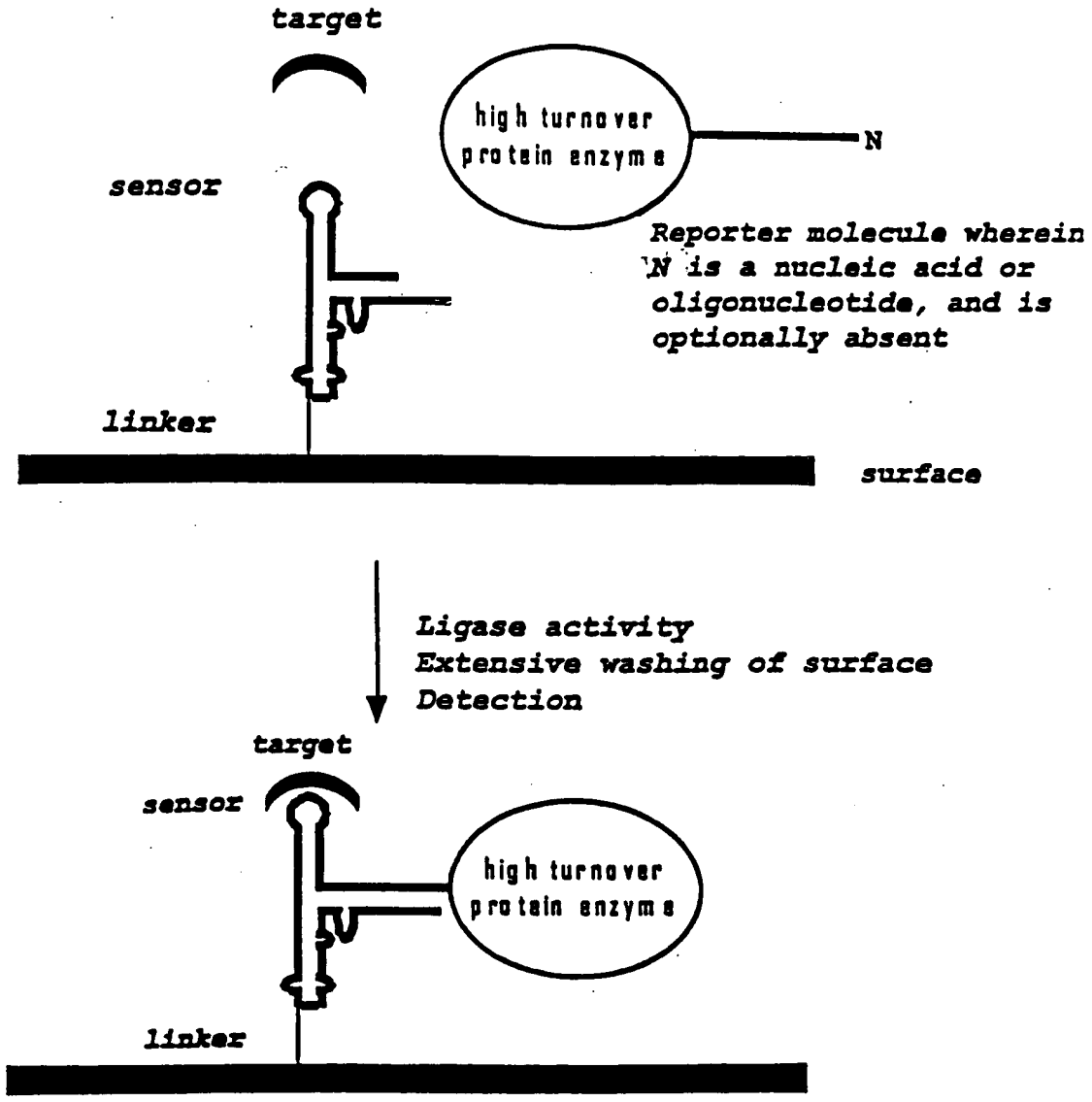
Figure 22: Amplification of Signal via use of protein enzyme conjugate



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Ligase Sensor Molecule with enzymatic reporter

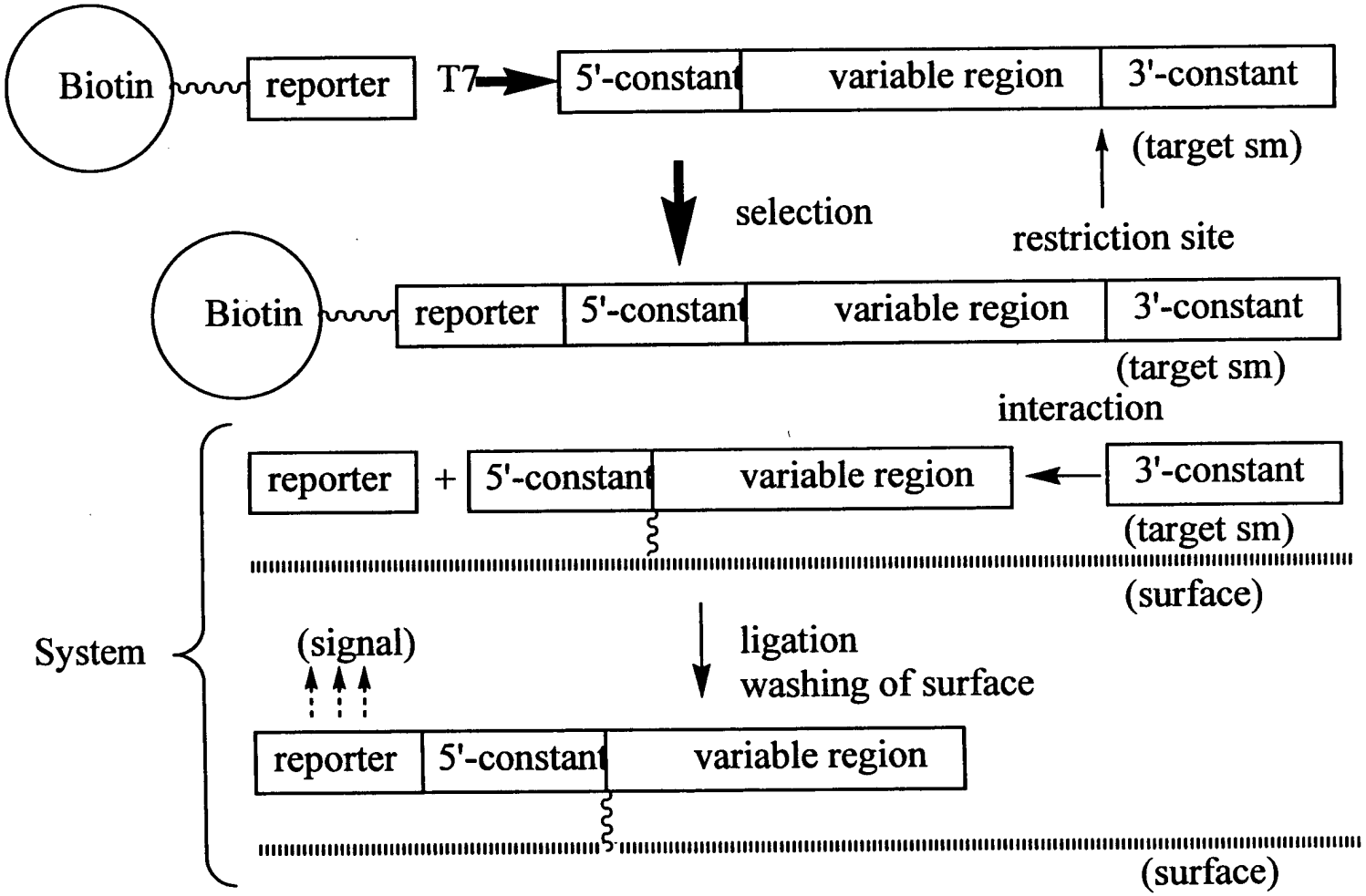


Alternatively, a fluorescent or chemiluminescent based reporter molecule is used.

FIG. 23

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Figure 24: Selection of Nucleic Acid Sensor Molecules with Ligase Activity

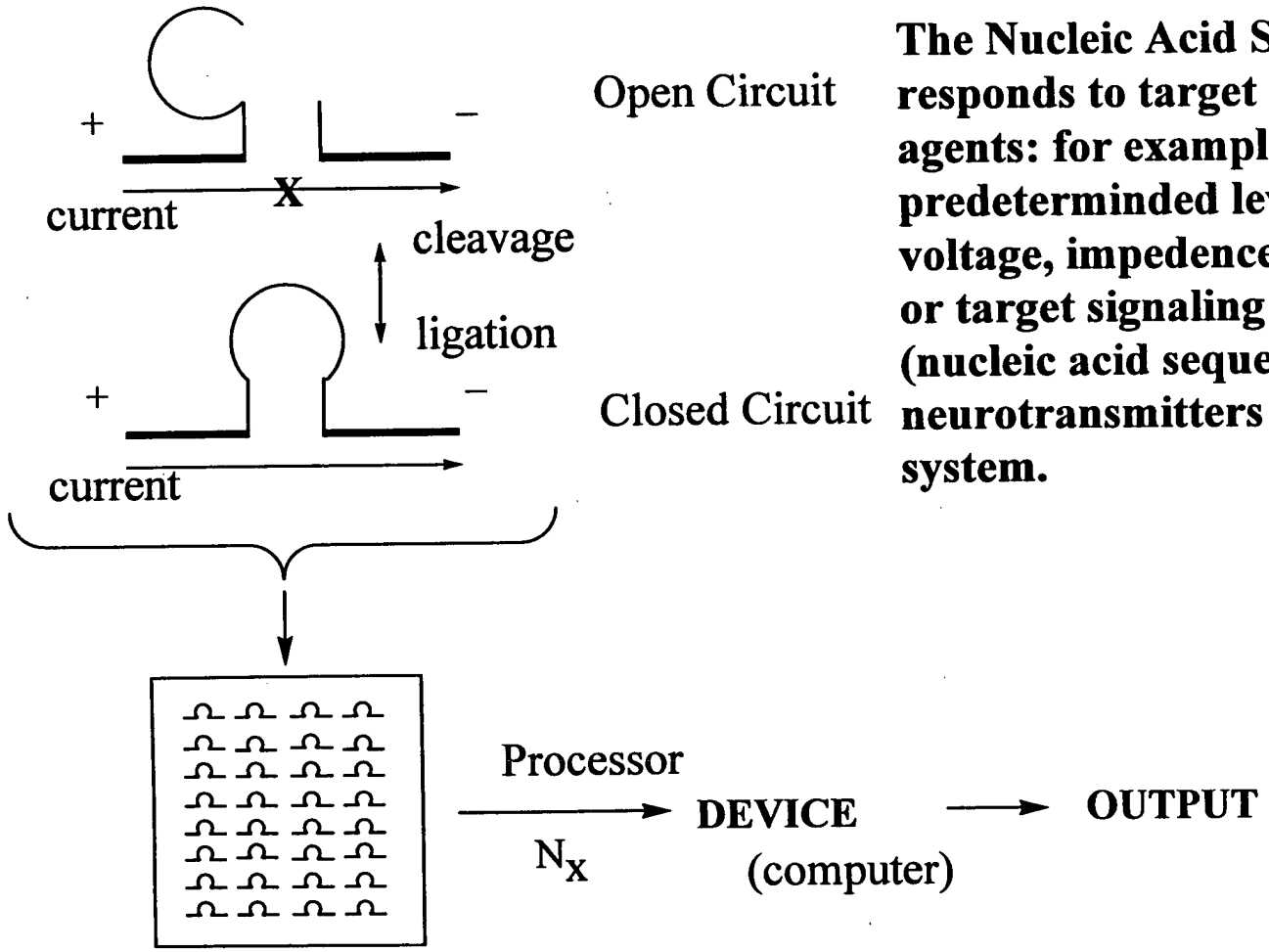


(sm= signaling molecule)

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Figure 25: Nucleic Acid Sensor Molecule-Based Electric Cirucuit



The Nucleic Acid Sensor Circuit responds to target signaling agents: for example predeterminded levels of current, voltage, impedence, or target signaling molecules (nucleic acid sequences, peptides, neurotransmitters etc.) in a system.

20E2TO" T9Z9500T

Figure 26: Target Inactivation of Zinzyme Sensor Molecule

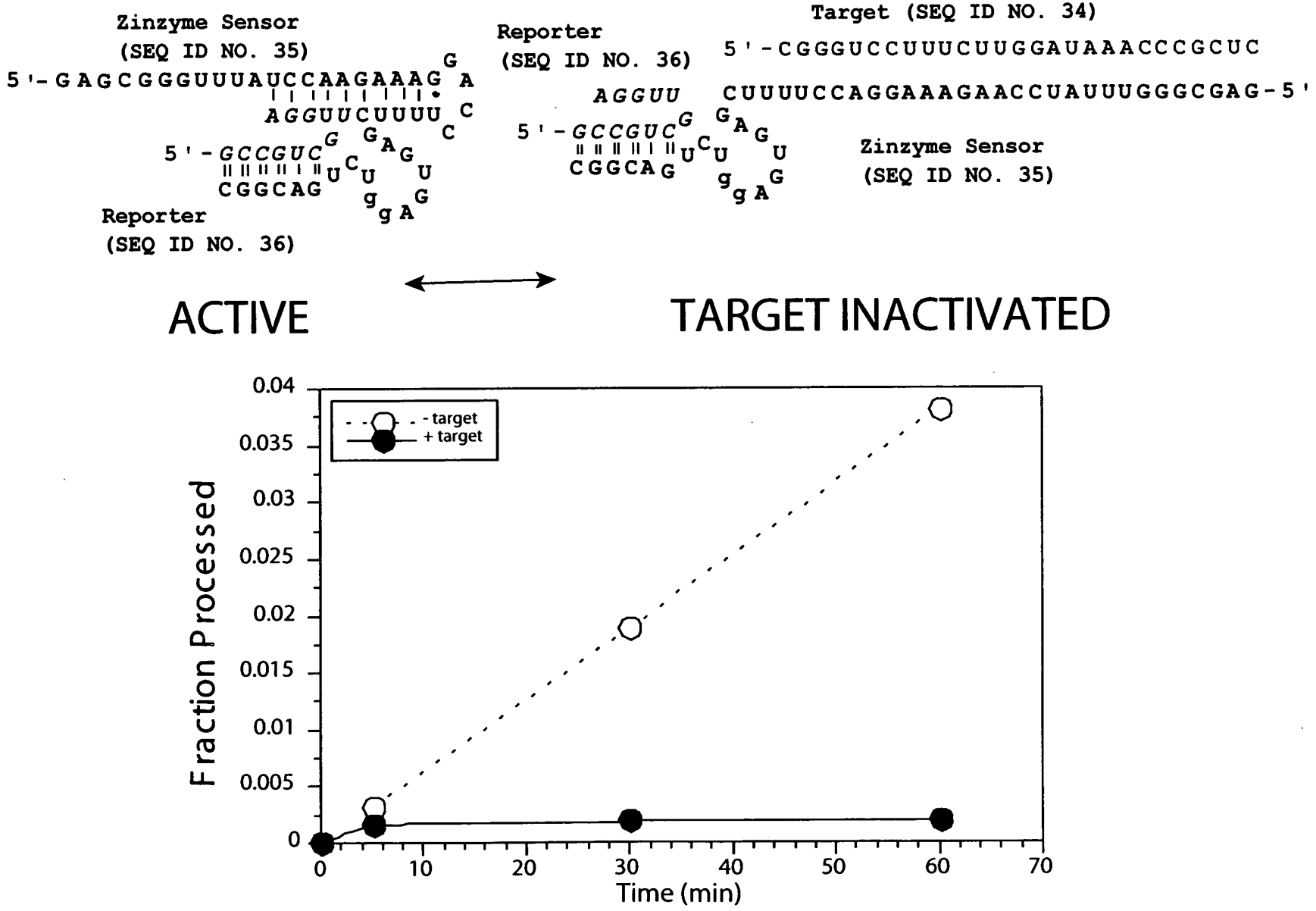
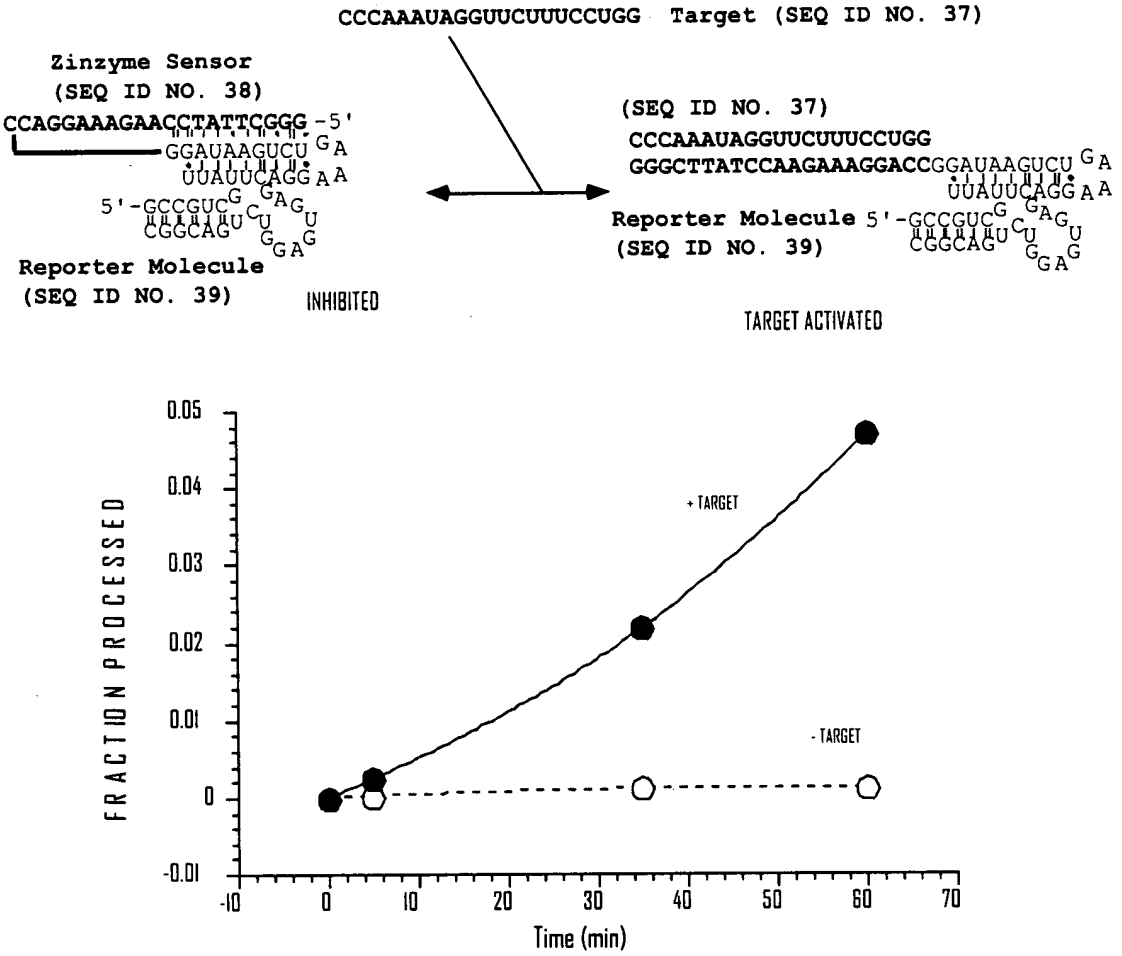


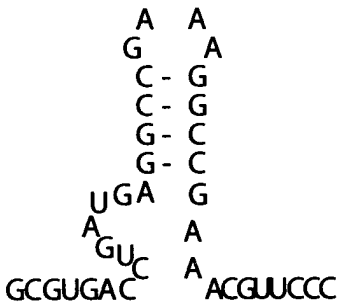
Figure 27: Target Activation of Zinzyme Sensor Molecule



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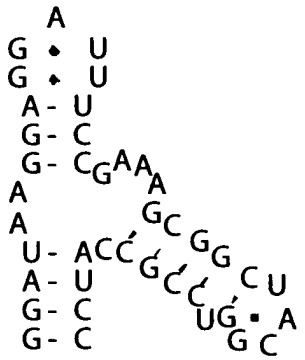
20E2TO" T9Z9500T

Figure 28: Erk modulated Nucleic Acid Sensor Molecule



HAMMERHEAD (HH)

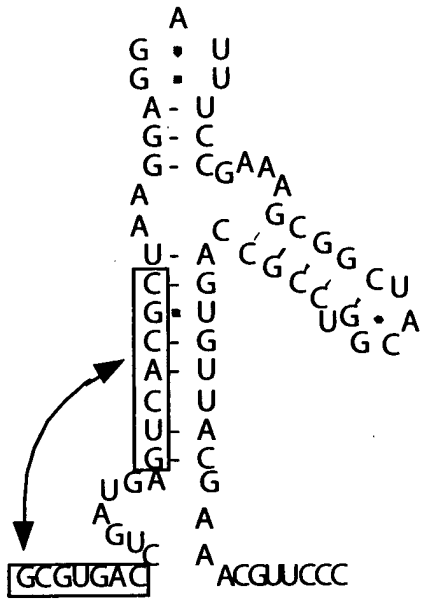
(SEQ ID NO. 40)



ERK SENSOR COMPONENT

K_D=300 pM

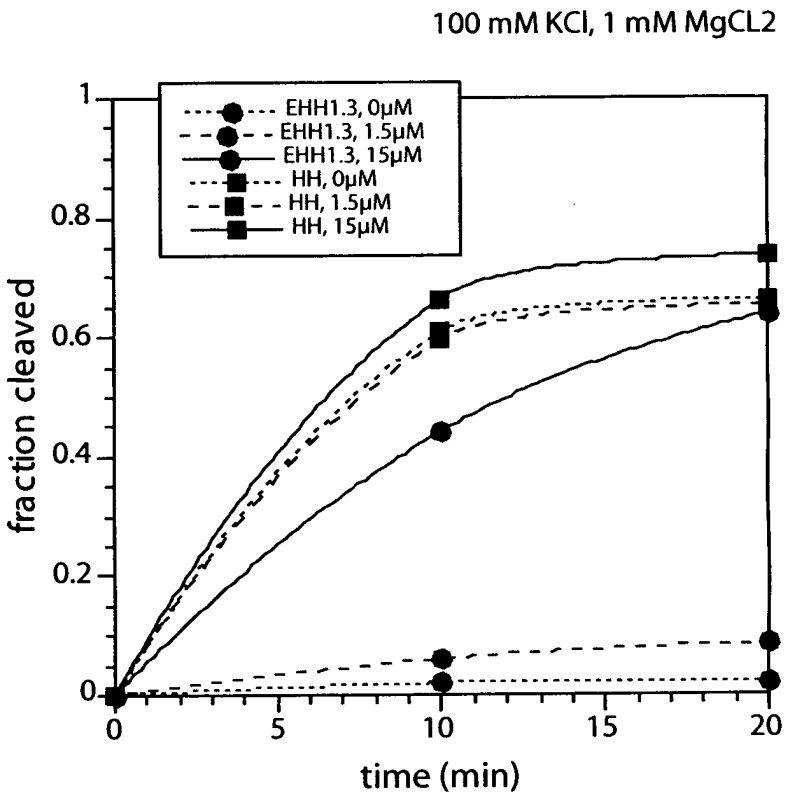
(SEQ ID NO. 41)



EHH1.3 sensor molecule

K_D=570 nM

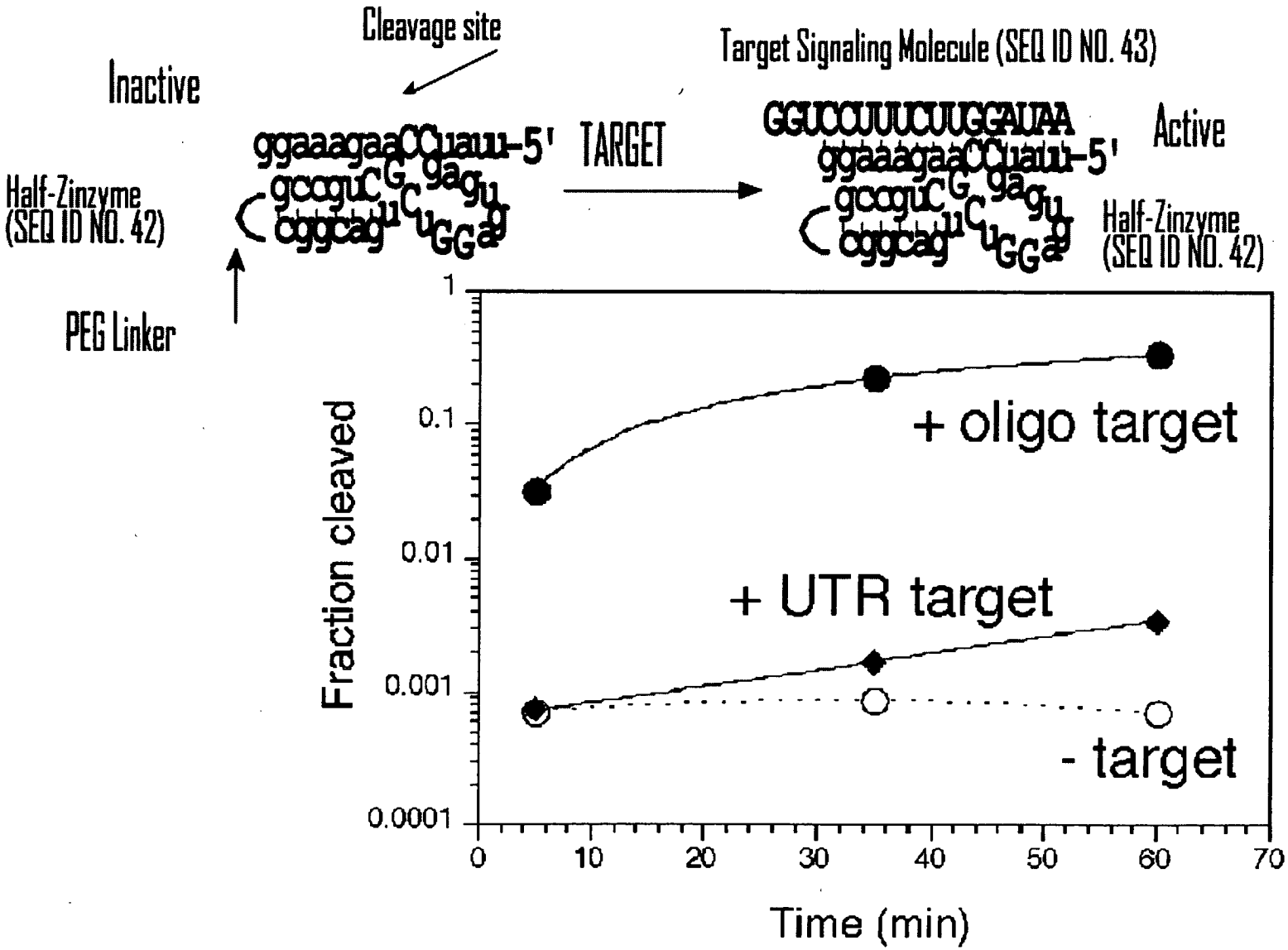
(SEQ ID NO. 42)



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Figure 29: Half-Zinzyme Construct

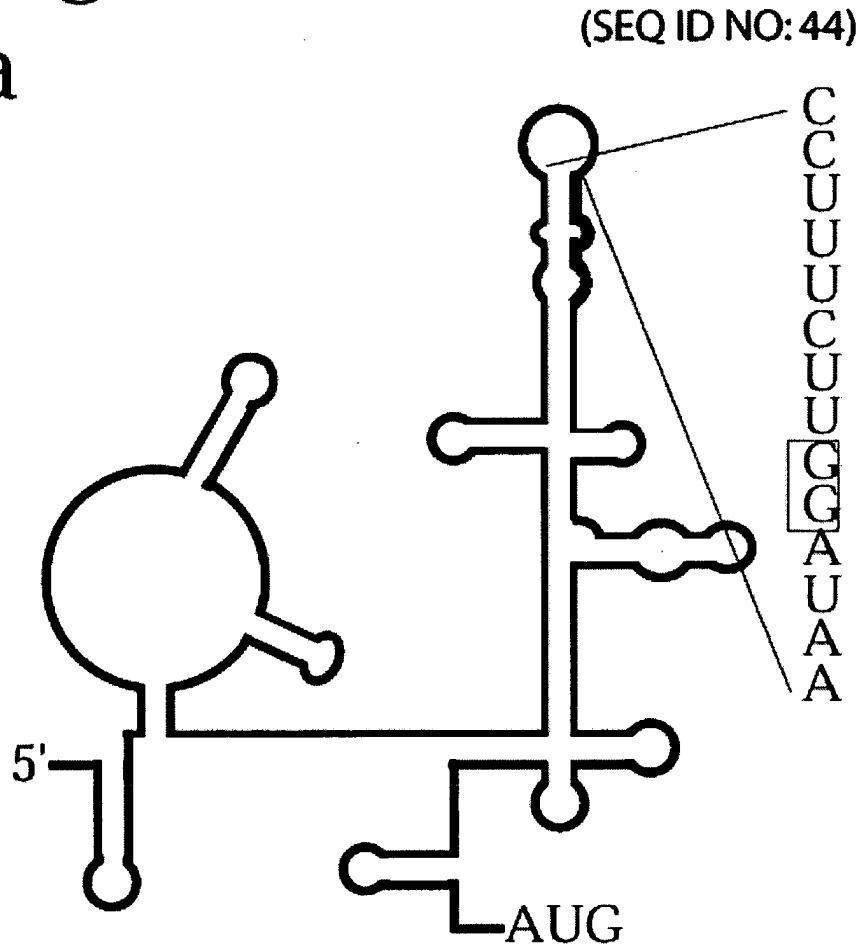


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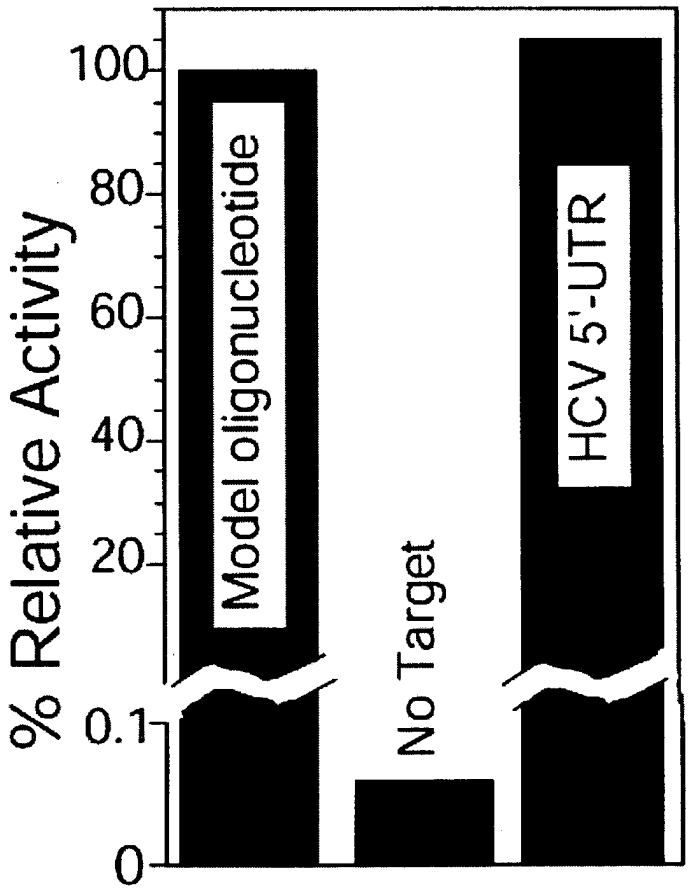
20E2TO" T9Z9SOOT

Figure 30

a

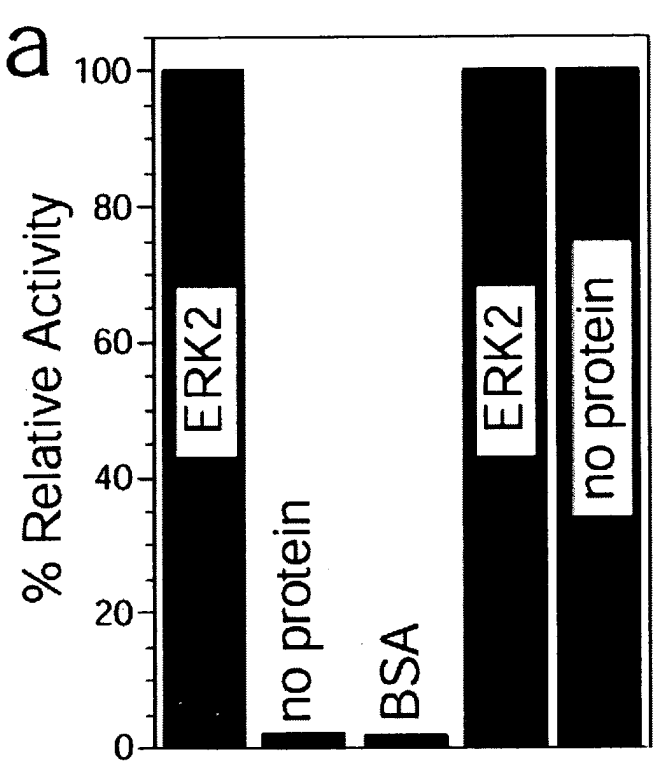


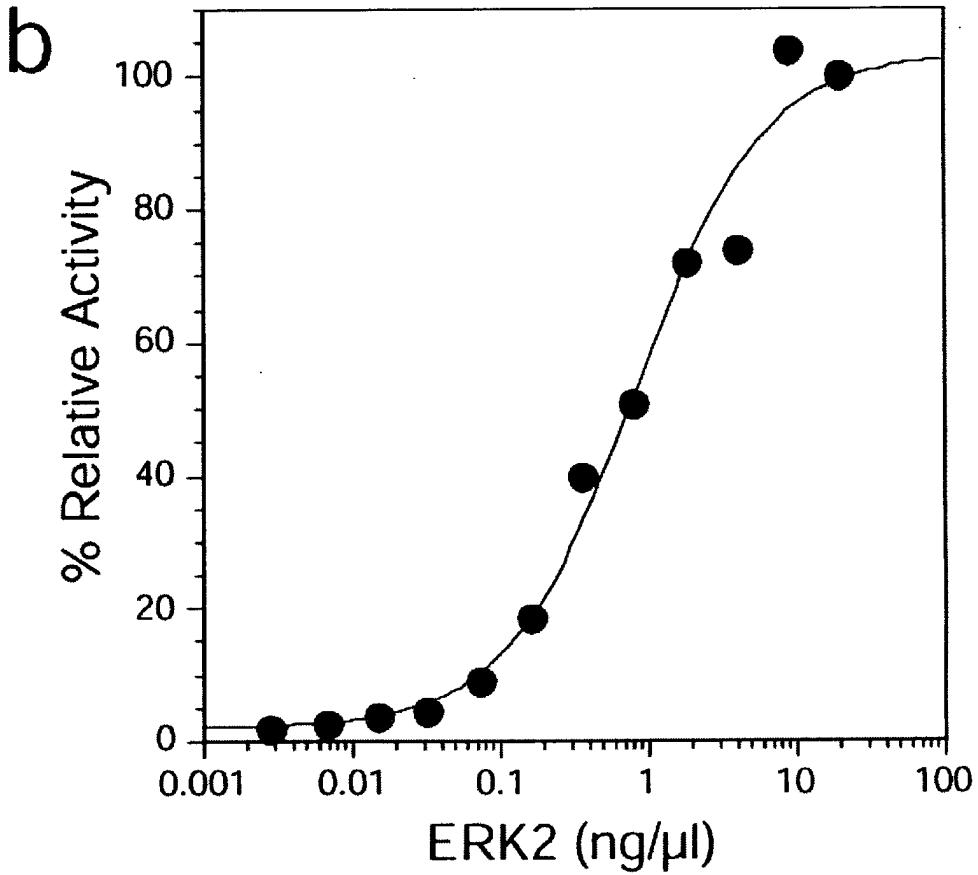
b



20020.T9795001

Figure 31





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20E2TO' T9Z9500T

Figure 32

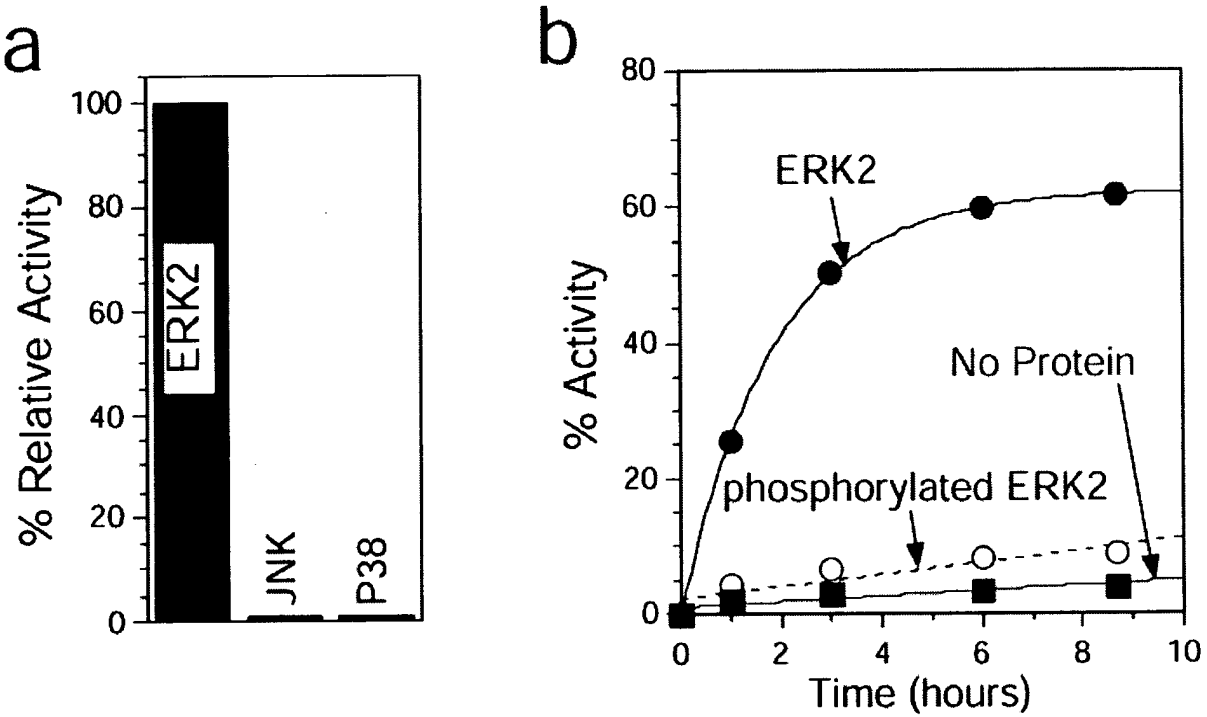
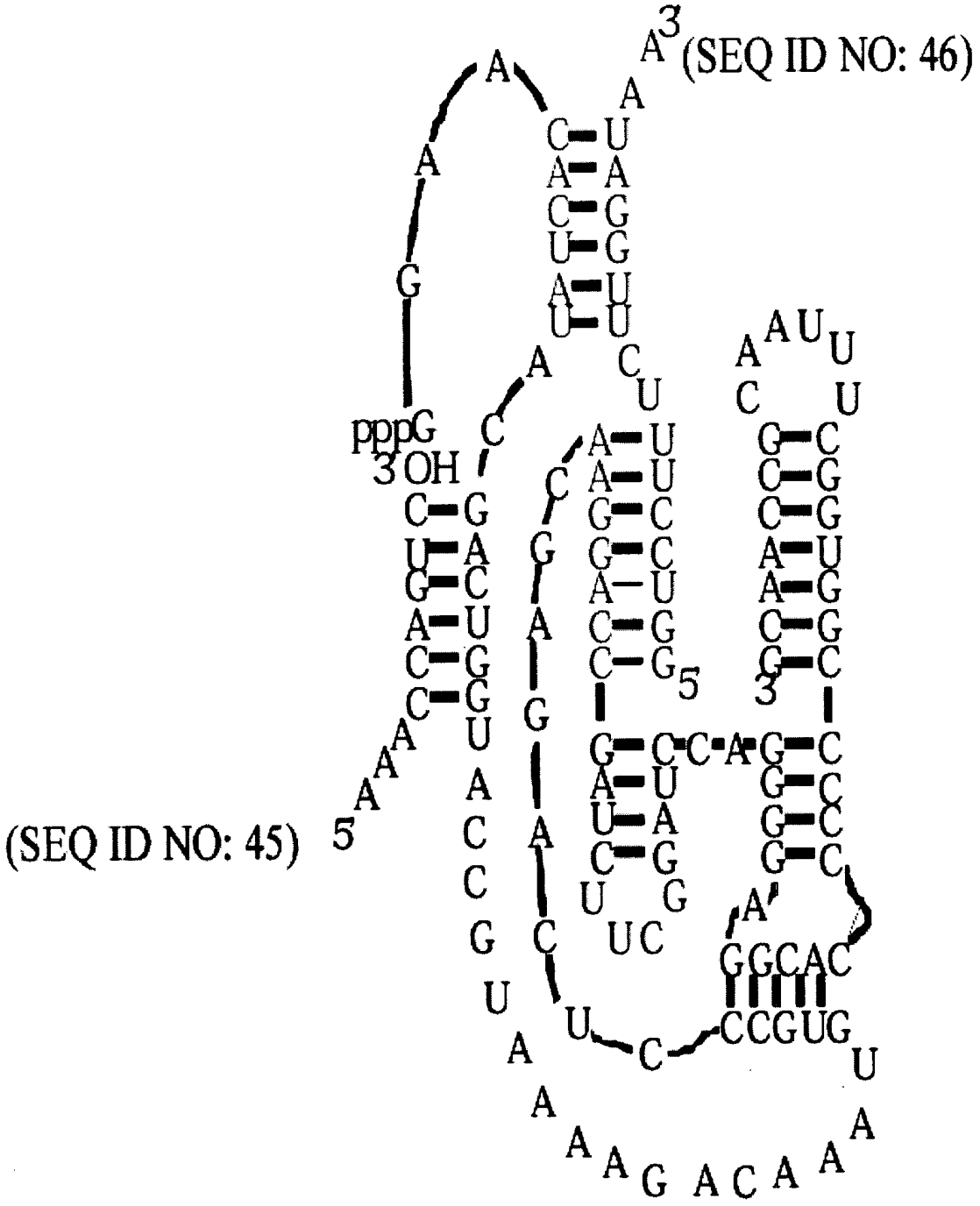


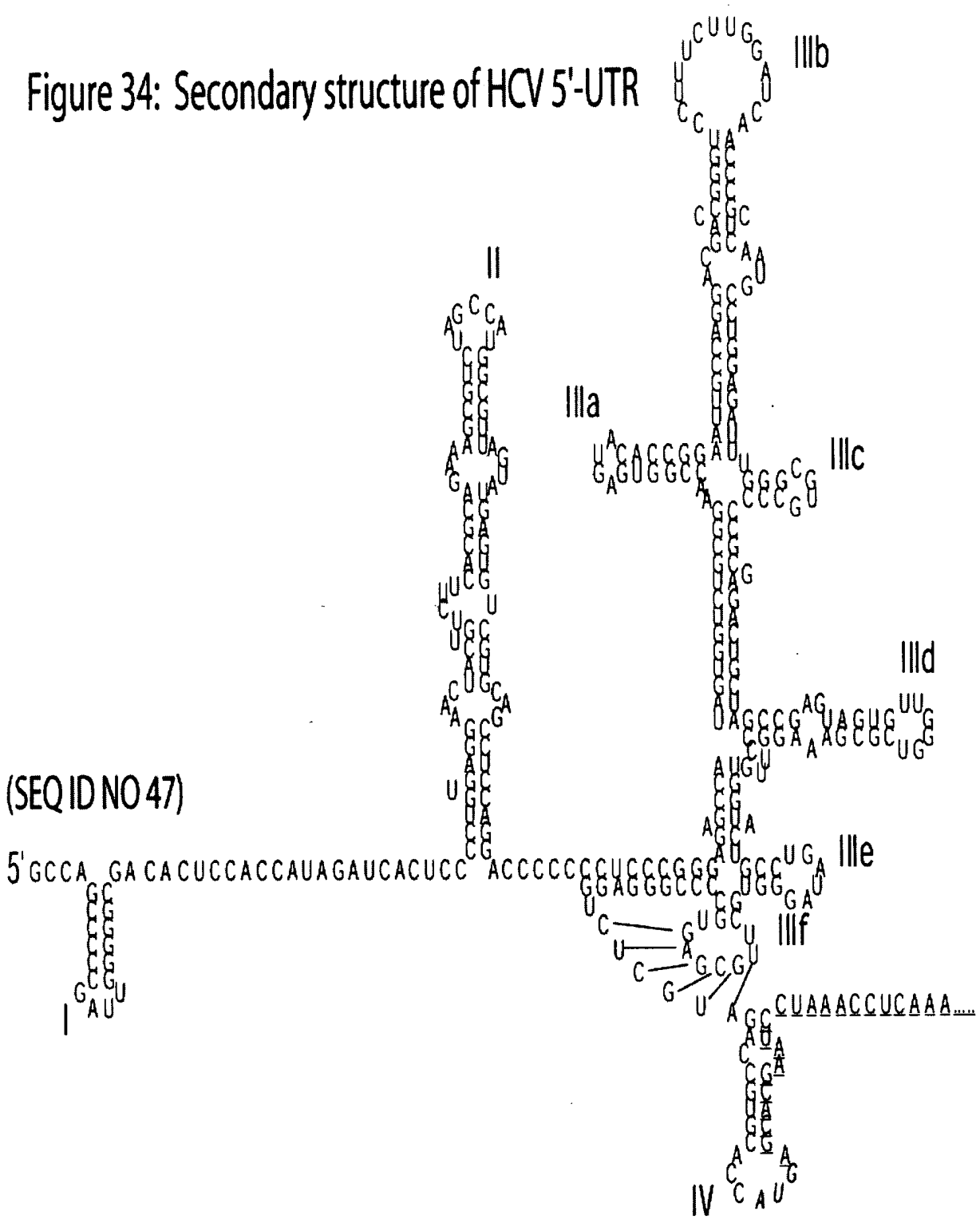
Figure 33: Halfzyme Ligase



4005676 - 052302

20030154005 • 08/14/2003

Figure 34: Secondary structure of HCV 5'-UTR



20E2TO" T9Z95OOT

Figure 35
Design of SNP Detection using Halfzyme-AZB7.1



		SEQ ID NO:
	5'-a g c g C c g a c g g g -3'	50
Target HBV 1887(True)=AZB7-GG	3'-T C G C **G** - **G** C T G C C C-5' (SNPT-1)	51
AZB7-AG	3'-T C G C **A** - **G** C T G C C C-5' (SNPT-2)	52
AZB7-TG	3'-T C G C **T** - **G** C T G C C C-5' (SNPT-3)	53
AZB7-CG	3'-T C G C **C** - **G** C T G C C C-5' (SNPT-4)	54
AZB7-GA	3'-T C G C **G** - **A** C T G C C C-5' (SNPT-5)	55
AZB7-GT	3'-T C G C **G** - **T** C T G C C C-5' (SNPT-6)	56
AZB7-GC	3'-T C G C **G** - **C** C T G C C C-5' (SNPT-7)	57
RNA HBV 1433	*3'-U C G C **G** - **G** C U G C C C-5'*	58

20020 T9795001

Figure 36: Single Nucleotide Polymorphism (SNP) Detection

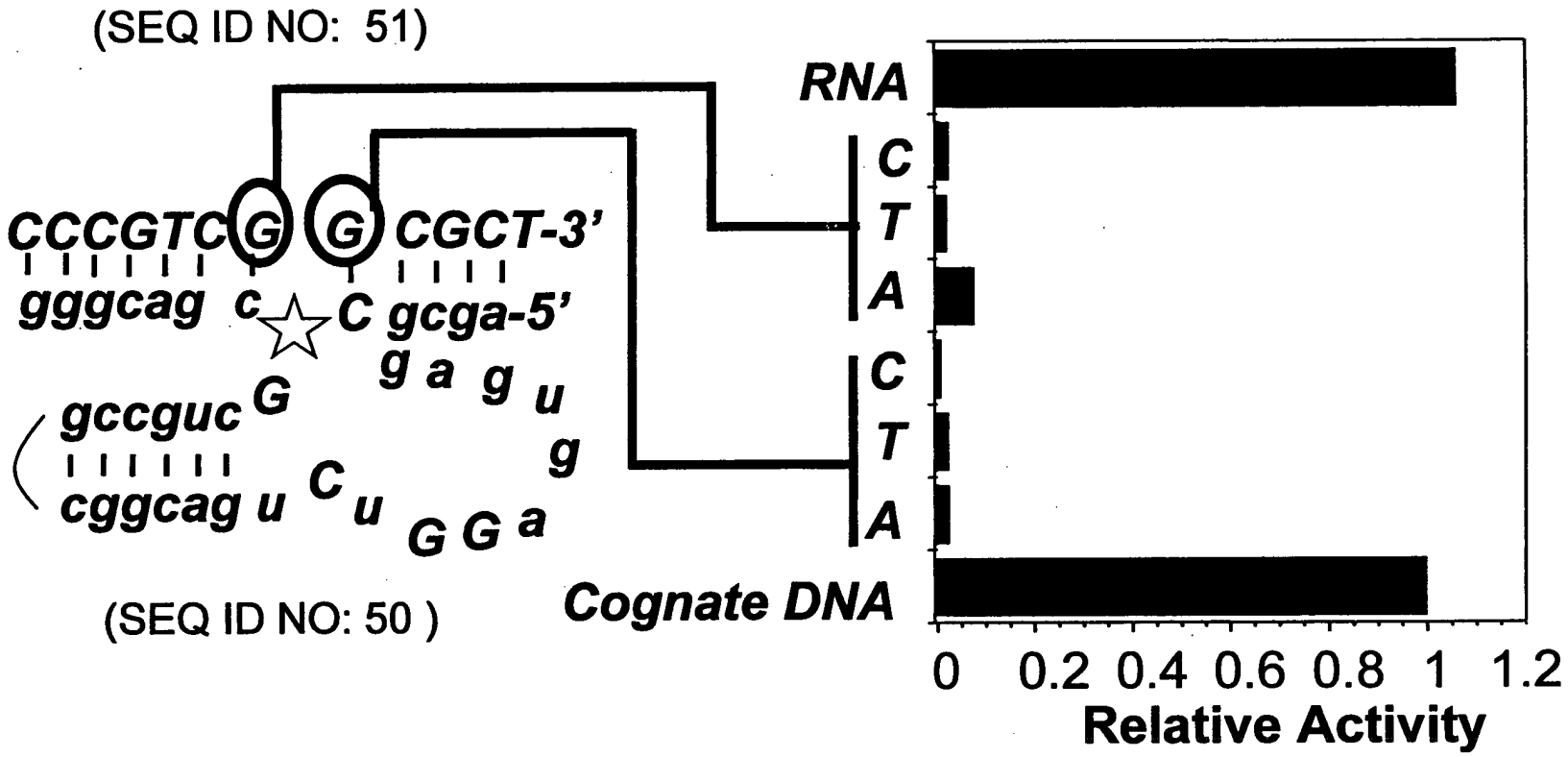
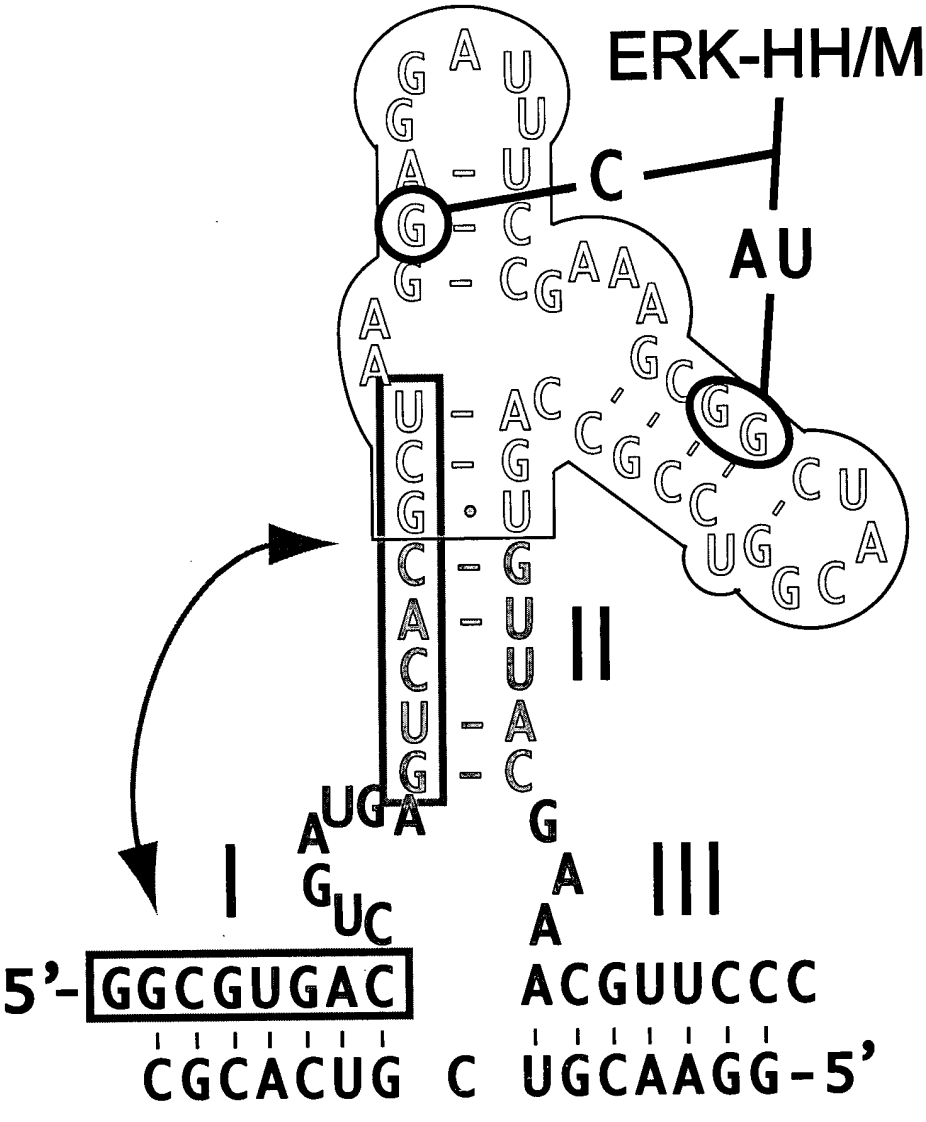


Figure 37A

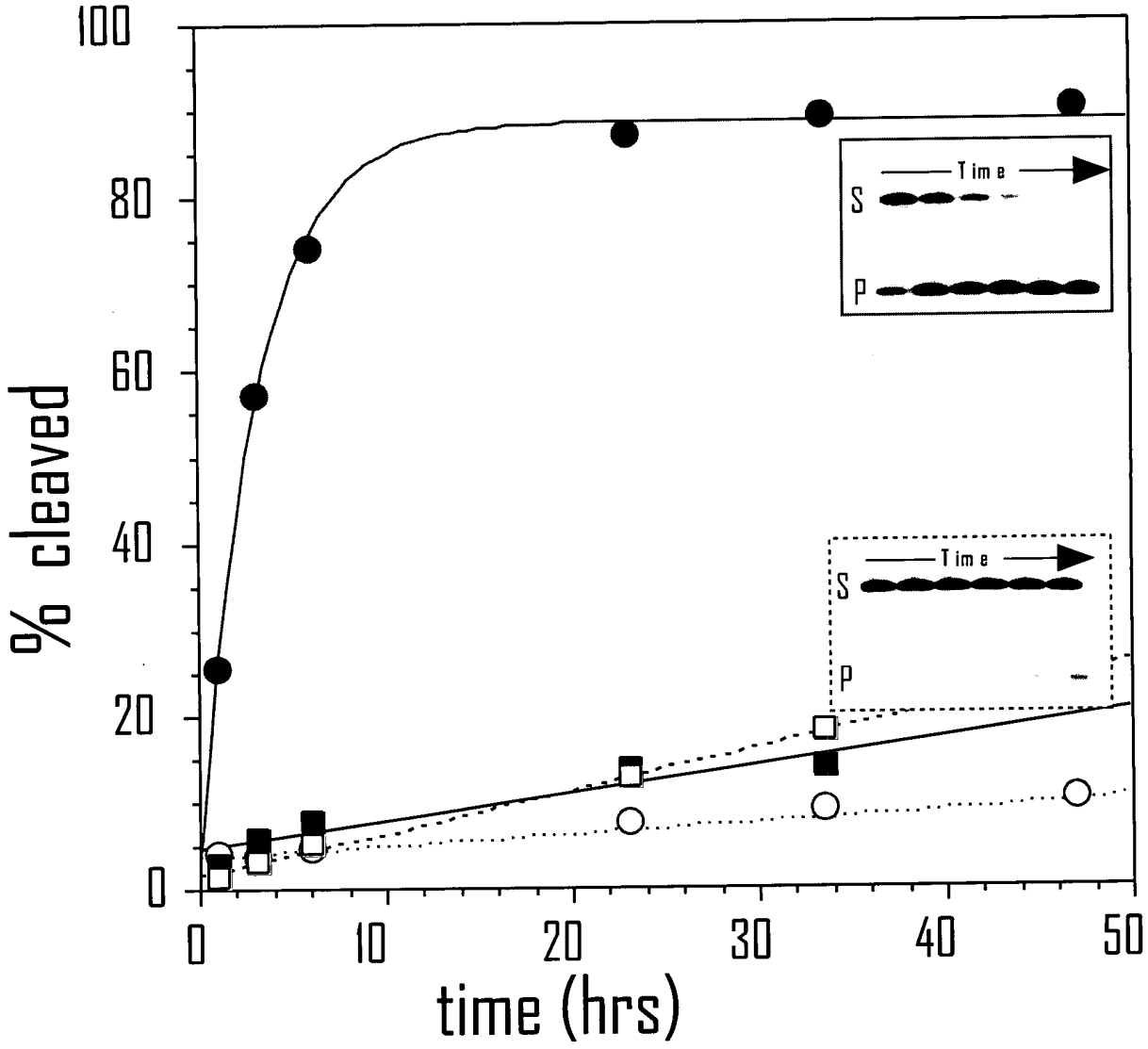


(SEQ ID NO: 61)

(SEQ ID NO: 62)

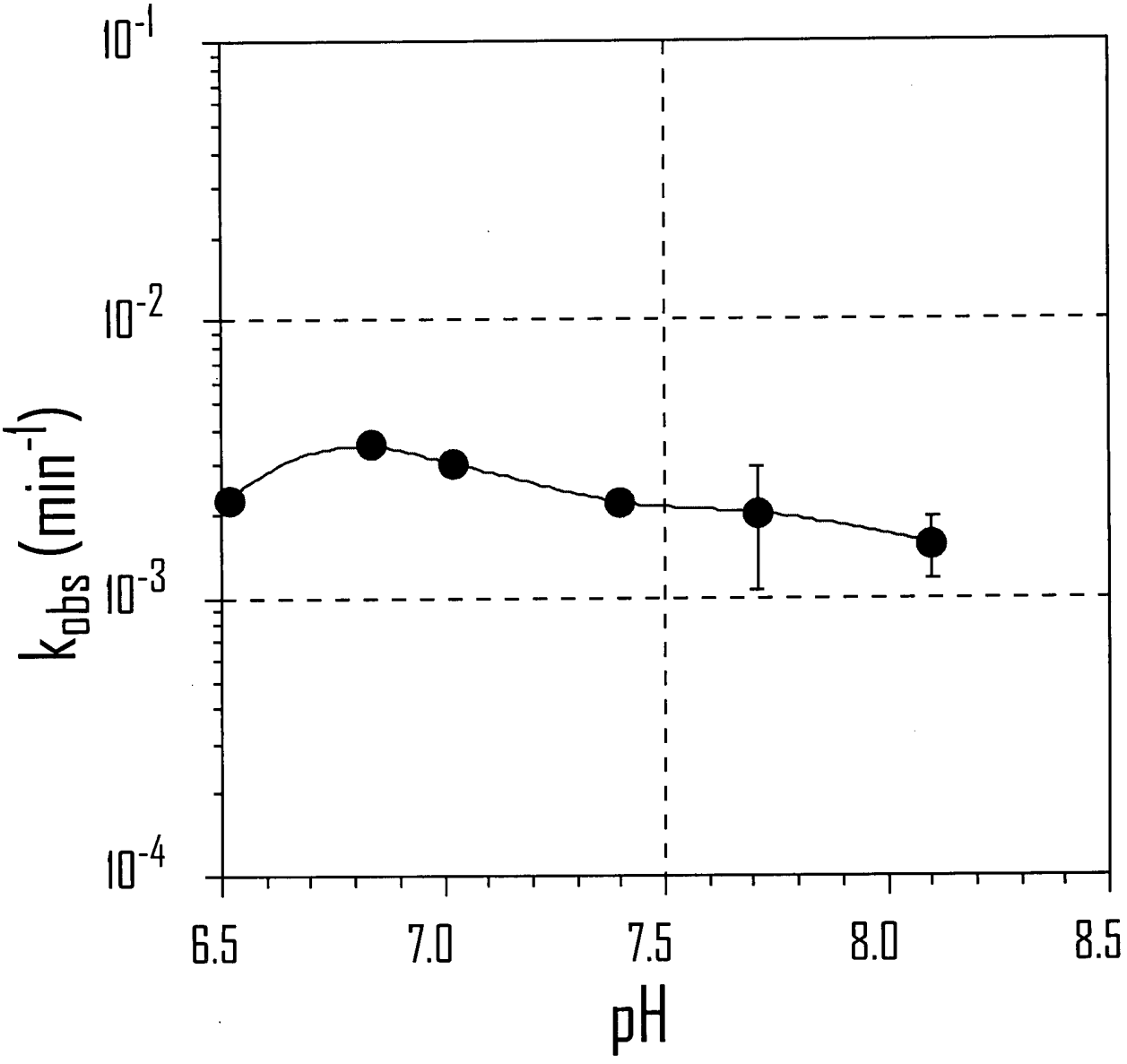
EDC3E3 .0T2S0T

Figure 37B



20030219.0230

Figure 37C



20020704.050

Figure 37D

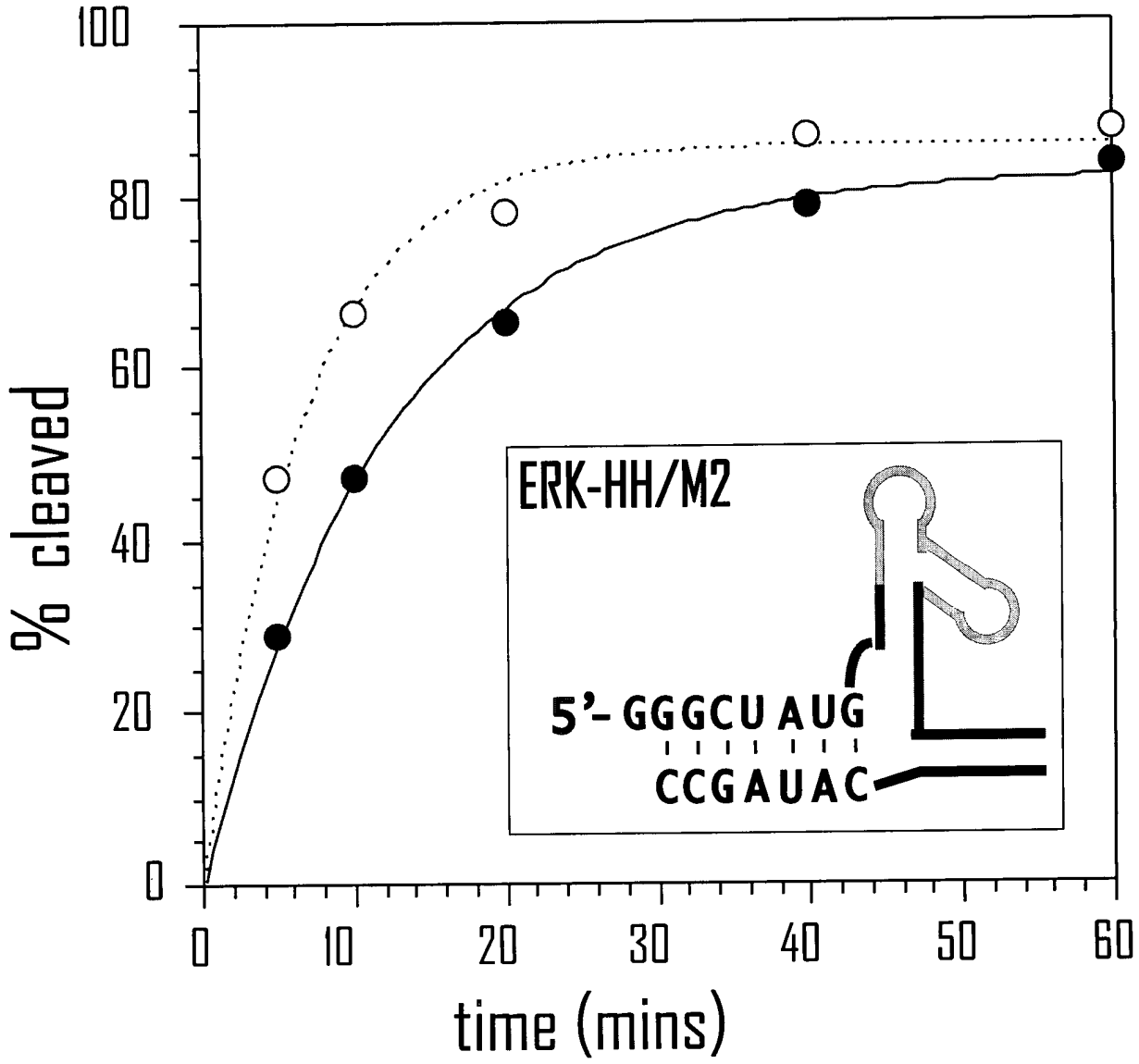

100
80
60
40
20
0
% cleaved
0
10
20
30
40
50
60
time (mins)
ERK-HH/M2
5'- GGGCU AUG
CCGAUAC

Figure 38

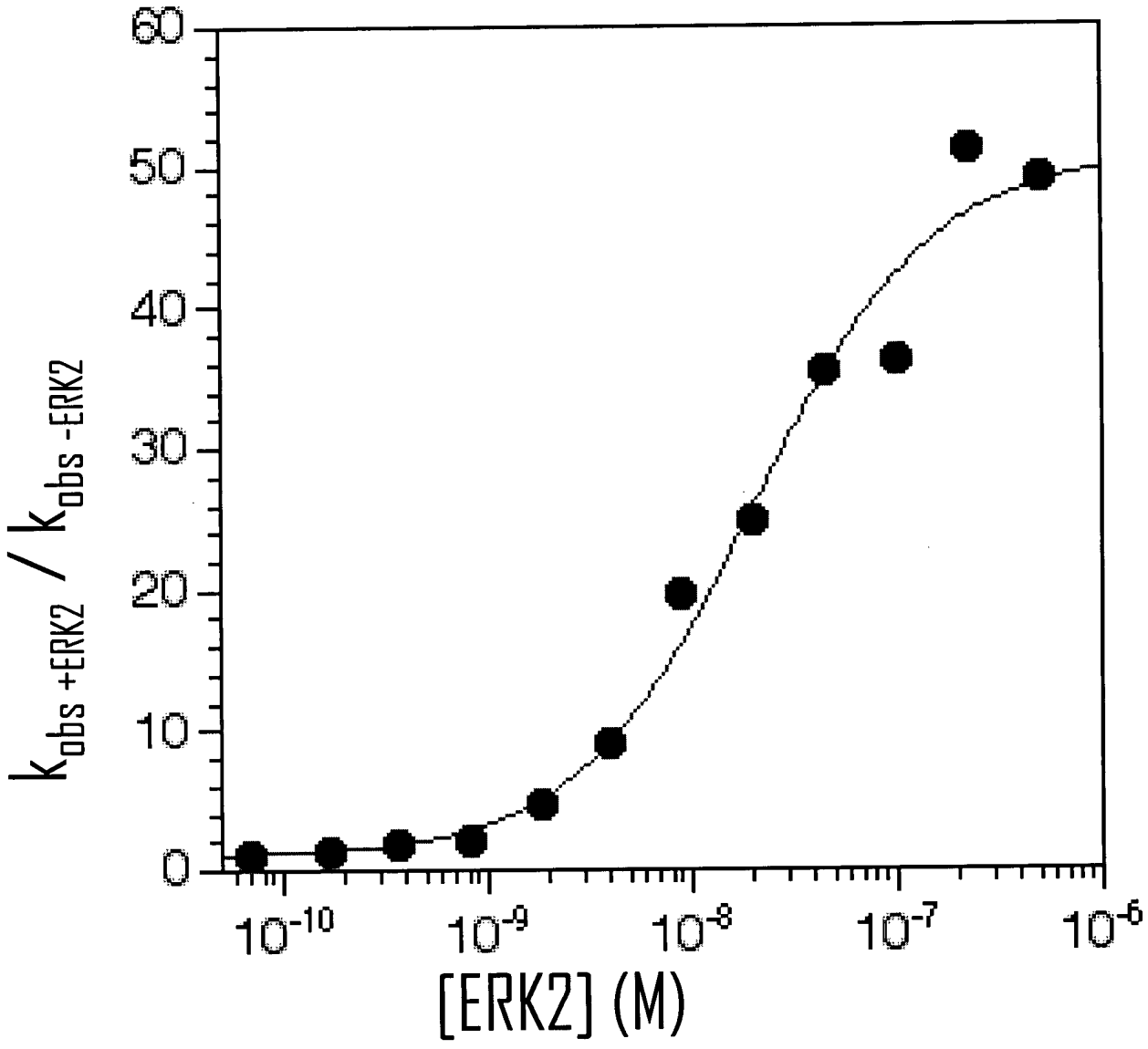
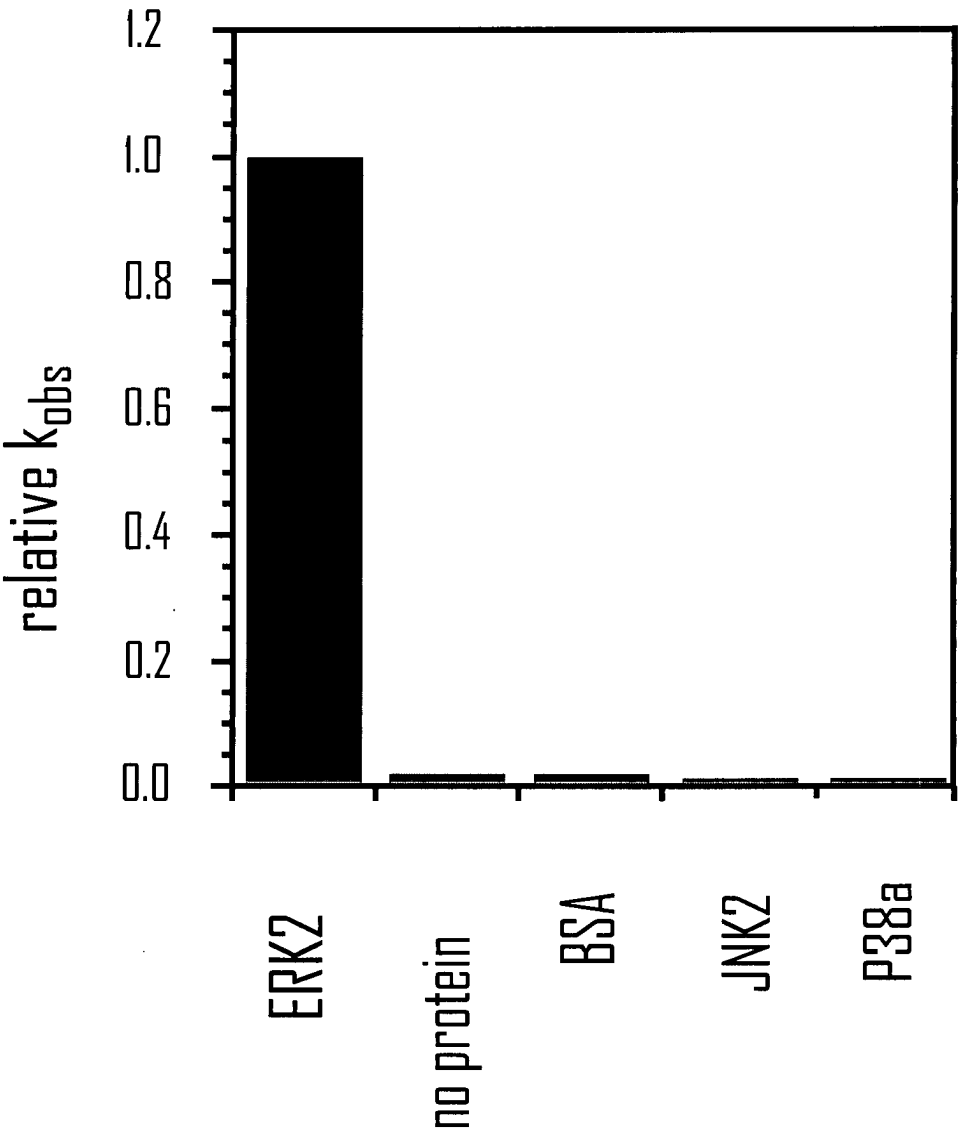
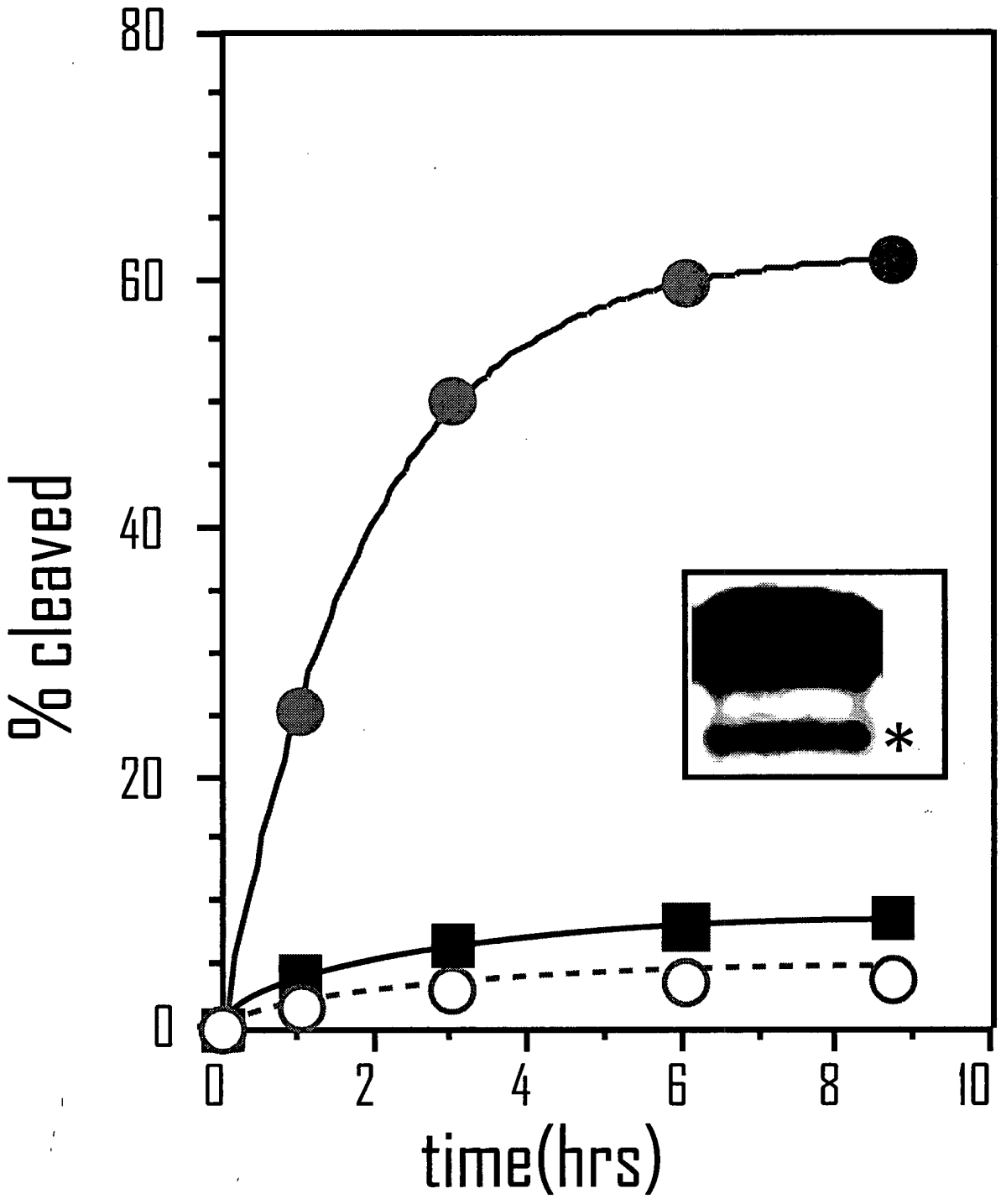

60
50
40
30
20
10
0
$k_{obs\ +ERK2}$ / $k_{obs\ -ERK2}$
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
[ERK2] (M)

Figure 39A



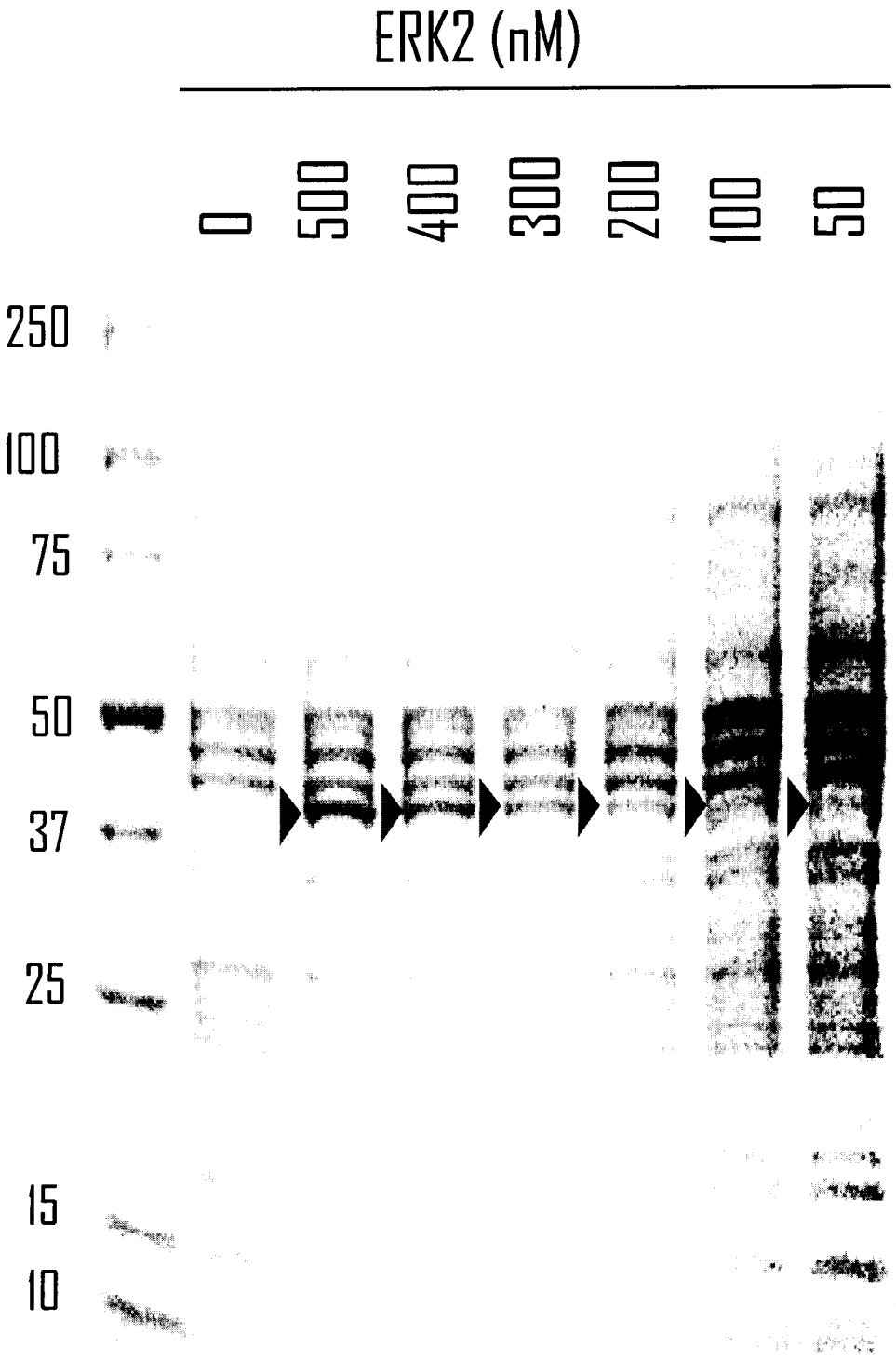
20020704 "Express Mail"

Figure 39B



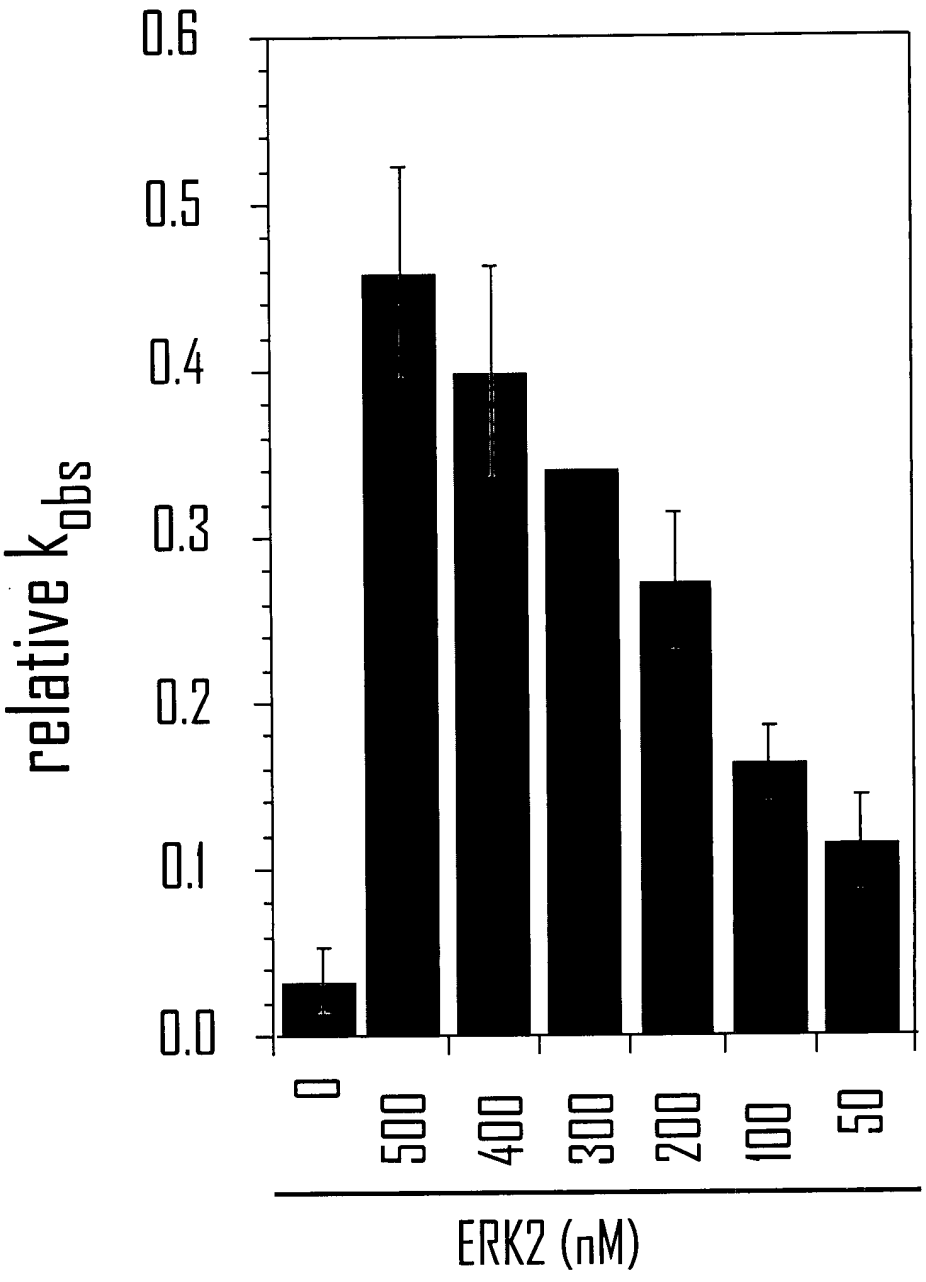
20020-034759.600

Figure 40A



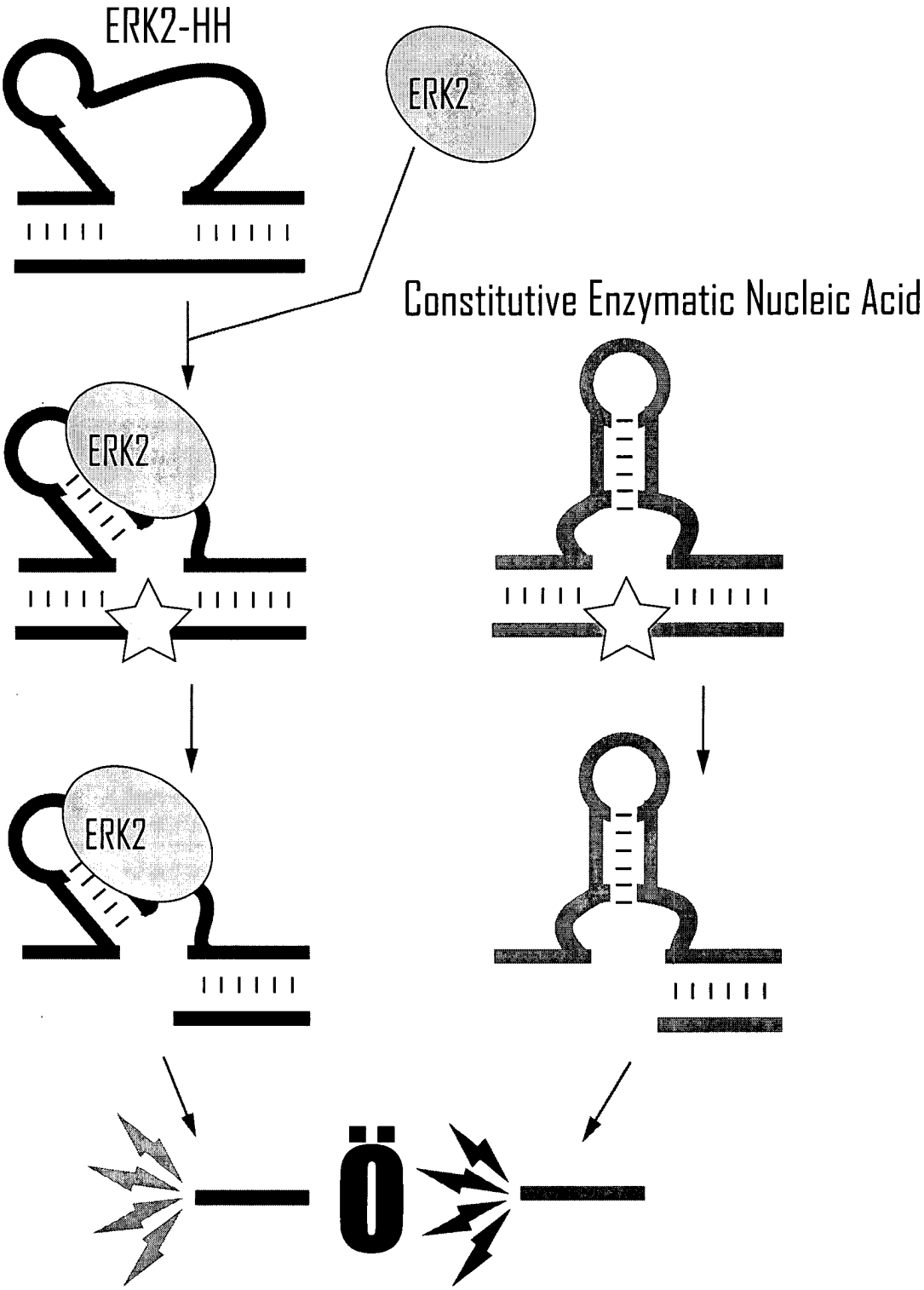
20020704.0950

Figure 40B



20030170.0125305

Figure 41A



10056761.012302

Figure 41B

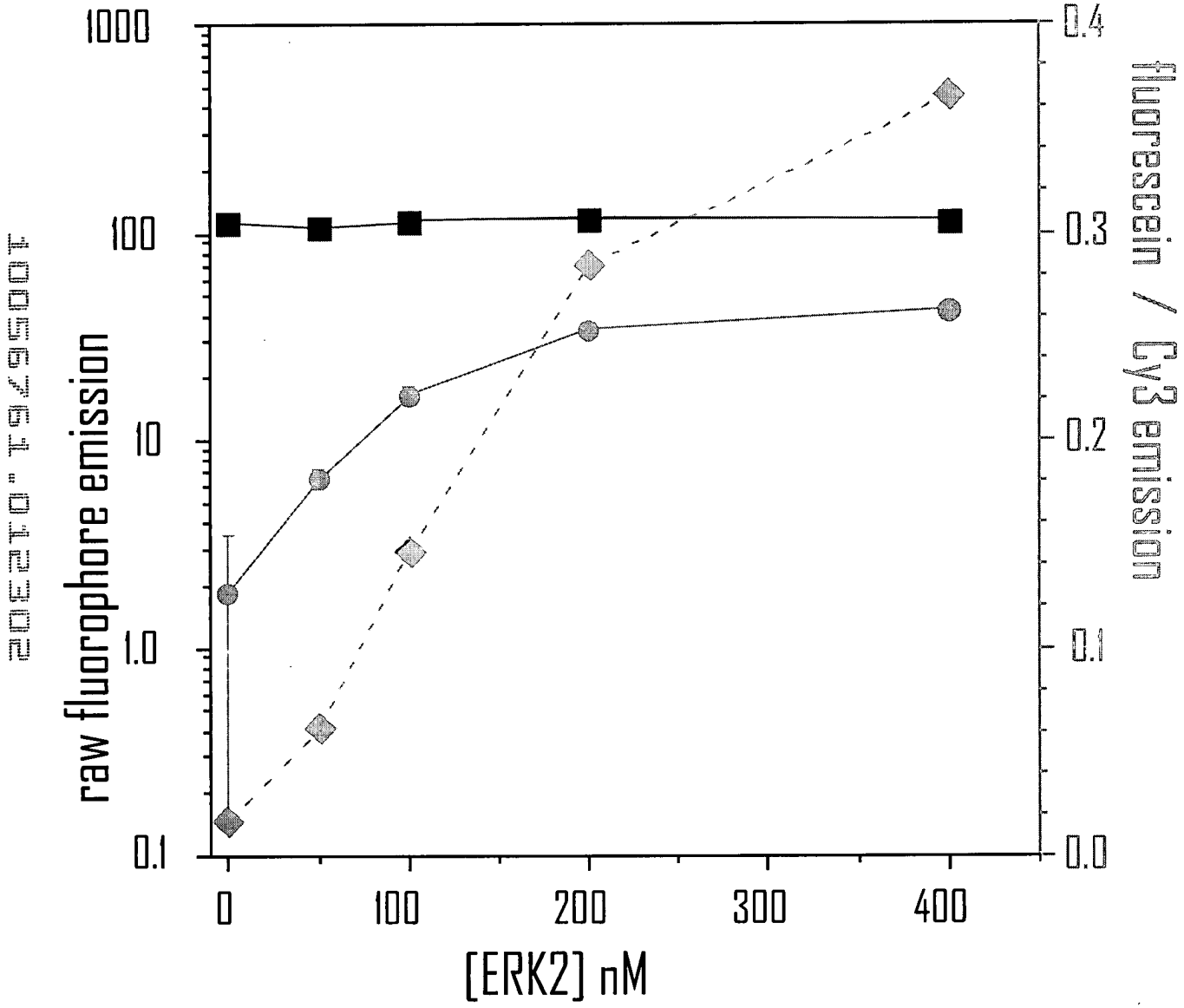
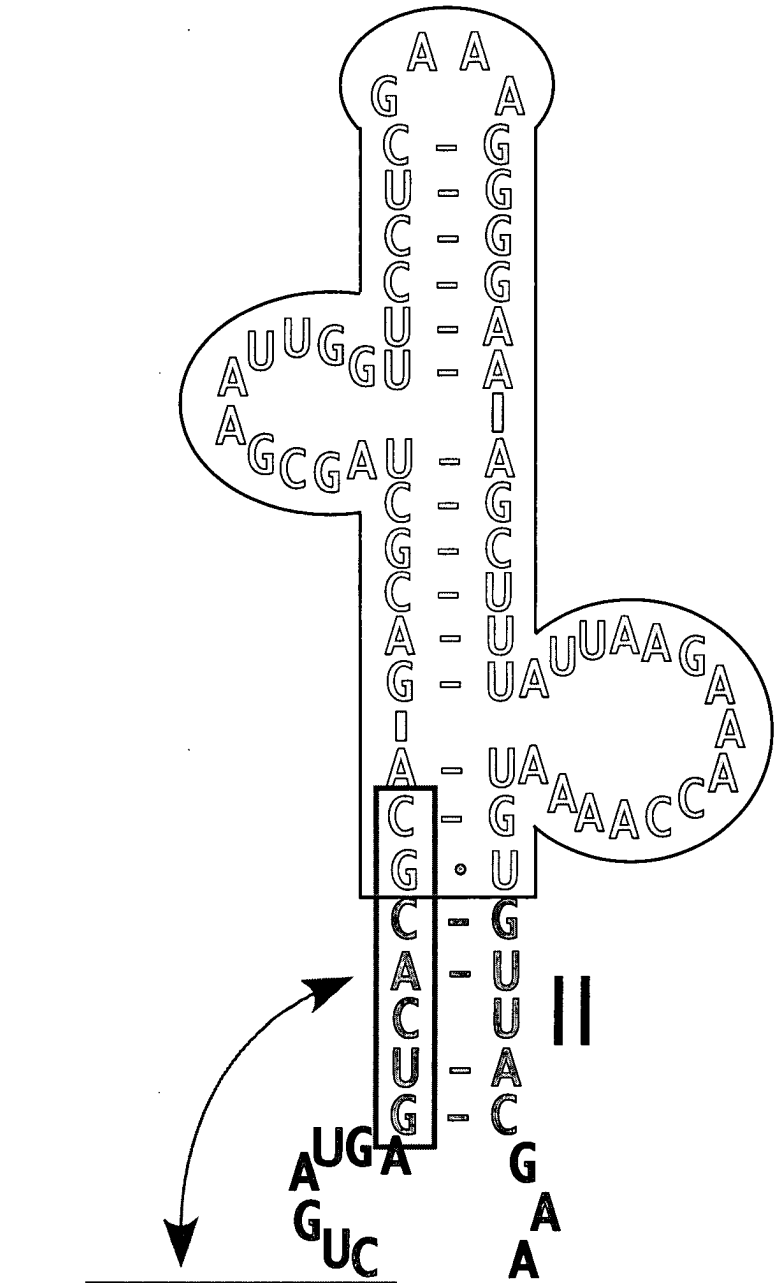

1000
100
10
1.0
0.1
raw fluorophore emission
0.4
0.3
0.2
0.1
0.0
fluorescein / Cy3 emission
0
100
200
300
400
[ERK2] nM

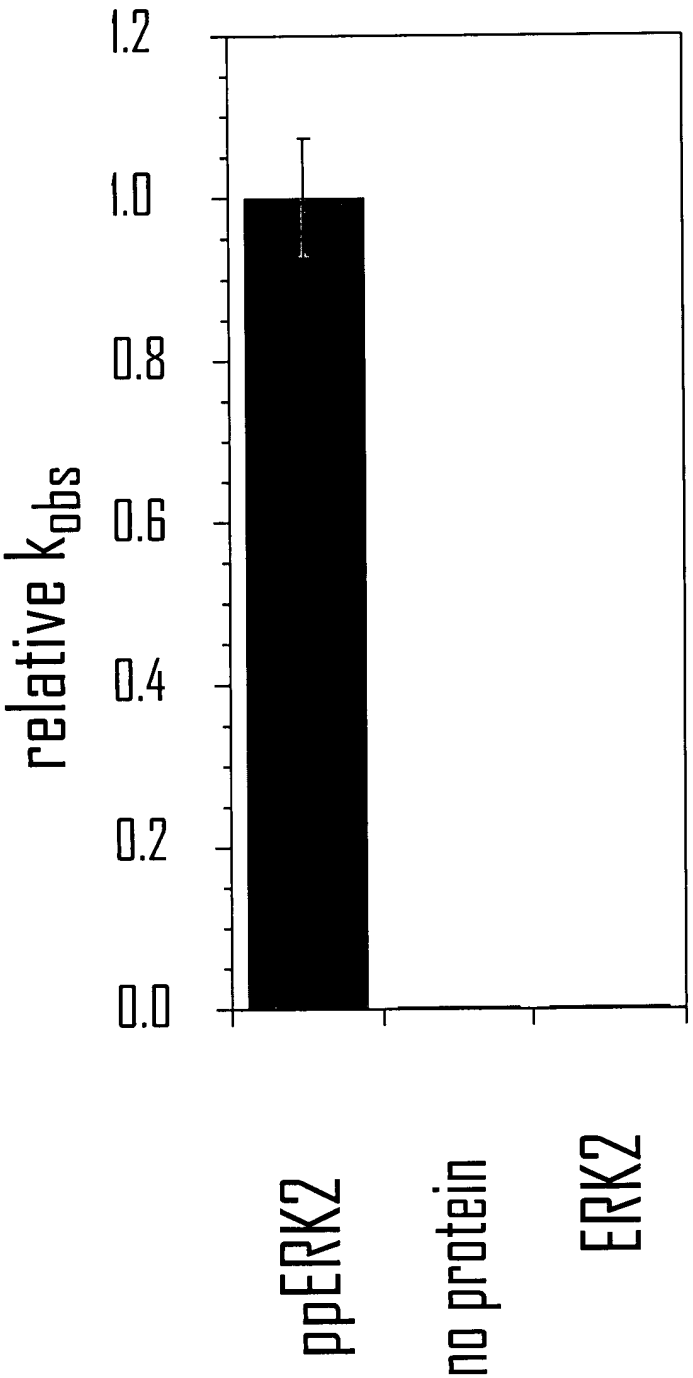
Figure 42A



5'-GGCGUGAC ACGUUCCC (SEQ ID NO: 63)
CGCACUG C UGCAAGG-5' (SEQ ID NO: 62)

20021202 1005576

Figure 42B



20030734.0056